Handbook of Experimental Pharmacology 242

Scott M. Whitcup
Dimitri T. Azar *Editors*

Pharmacologic Therapy of Ocular Disease

Springer

Handbook of Experimental Pharmacology

Volume 242

More information about this series at http://www.springer.com/series/164

Scott M. Whitcup • Dimitri T. Azar
Editors

Pharmacologic Therapy of Ocular Disease

Springer

Editors
Scott M. Whitcup
Akrivista and Whitecap Biosciences
Mission Viejo, California
USA

Jules Stein Eye Institute
David Geffen School of Medicine
at UCLA
Los Angeles, California
USA

Dimitri T. Azar
Illinois Eye and Ear Infirmary
University of Illinois at Chicago
College of Medicine
Chicago, Illinois
USA

ISSN 0171-2004 ISSN 1865-0325 (electronic)
Handbook of Experimental Pharmacology
ISBN 978-3-319-58288-7 ISBN 978-3-319-58290-0 (eBook)
DOI 10.1007/978-3-319-58290-0

Library of Congress Control Number: 2017941200

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Louis Pasteur said, "There are no such things as applied sciences, only applications of science." The application of scientific advancements over the last two decades has led to a plethora of new sight-saving therapies. It is both illuminating and encouraging to compare the last volume of the *Handbook of Experimental Pharmacology* devoted to the *Pharmacology of the Eye* published in 1984 to the current volume. The 1984 version, edited by Marvin Sears, provided a sound physiologic and pharmacologic foundation to guide ophthalmic therapy. The medications that were discussed focused on glaucoma, ocular infections, and ocular inflammatory disease. There was little discussion of therapies for retinal diseases or dry eye, and topics such as sustained-release drug delivery were not discussed.

The goal of the current volume is to present the science that can further catalyze the progress in ocular pharmacology as well as to review the resulting new therapies available today and in future trials. Chapters on ocular pharmacology and ocular pharmacokinetics are presented at the start of the book and provide the principles forming the basis of the subsequent disease-focused chapters spanning the tear film to the optic nerve and tissues in between.

Some of the new therapies discussed in this volume include new classes of drugs to treat ocular hypertension and glaucoma, anti-VEGF therapy for retinal disease, and the use of biologic agents to manage ocular inflammatory diseases. A chapter on drug delivery has been included since extendedrelease of medications will be required to improve patient care and allow the practicaladministration of certain compounds to the eye. Discussion of gene therapy for diseases such as retinitis pigmentosa and neuroprotection for diseases of the optic nerve are also recent additions.

Winston Churchill suggested that, "if you have knowledge, let others light their candles with it." It is our hope that this book will serve not only to summarize the current state of the science of ocular pharmacology and therapeutics, but also to stimulate and support further advances that will benefit patients.

We would like to thank the authors for their excellent contributions and for their dedications. Our thanks also go to Sumathy Thanigaivelu, Suzanne Dathe, and Balamurugan Elumalai at Springer, and to Dr. Joelle Hallak for their tireless efforts

in making this project possible. Finally, we would like to dedicate this book to Dr. Robert Nussenblatt, who contributed to both this edition and the volume published in 1984. Bob was a teacher, mentor, and friend, and worked throughout his career to improve patient care.

Mission Viejo, CA, USA — Scott M. Whitcup
Chicago, IL, USA — Dimitri T. Azar

Contents

Principles of Ocular Pharmacology

Yong Park, Dorette Ellis, Brett Mueller, Dorota Stankowska, and Thomas Yorio

Contents

Abstract

Recently, in a poll by Research America, a significant number of individuals placed losing their eyesight as having the greatest impact on their lives more so than other conditions, such as limb loss or memory loss. When they were also

Y. Park • D. Ellis • D. Stankowska • T. Yorio (✉)
North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX, USA
e-mail: Thomas.Yorio@unthsc.edu

B. Mueller
Department of Ophthalmology, University of Louisville, Louisville, KY, USA

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_25

asked to rank which is the worst disease that could happen to them, blindness was ranked first by African-Americans and second by Caucasians, Hispanics, and Asians. Therefore, understanding the mechanisms of disease progression in the eye is extremely important if we want to make a difference in people's lives. In addition, developing treatment programs for these various diseases that could affect our eyesight is also critical. One of the most effective treatments we have is in the development of specific drugs that can be used to target various components of the mechanisms that lead to ocular disease. Understanding basic principles of the pharmacology of the eye is important if one seeks to develop effective treatments. As our population ages, the incidence of devastating eye diseases increases. It has been estimated that more than 65 million people suffer from glaucoma worldwide (Quigley and Broman. Br J Ophthalmol 90:262–267, 2006). Add to this the debilitating eye diseases of age-related macular degeneration, diabetic retinopathy, and cataract, the number of people effected exceeds 100 million. This chapter focuses on ocular pharmacology with specific emphasis on basic principles and outlining where in the various ocular sites are drug targets currently in use with effective drugs but also on future drug targets.

Keywords
Ocular drugs • Ocular Pharmacology • Pharmacodynamics

1 Introduction

We will discuss the pharmacodynamics and the pharmacokinetic properties of ocular therapeutic agents. Pharmacokinetics is the study of drug absorption, distribution, metabolism, and excretion of drugs. Clinical pharmacokinetics applies the data gathered in these studies to design optimal dosing and minimize adverse reactions for optimal therapeutic outcomes. In general, for drugs to reach their target organs (distribution), they first need to enter into the system circulation. Drugs given orally, intramuscularly, or by transdermal patch need to be absorbed through several physiological barriers before reaching the systemic circulation. Drugs are metabolized by specific enzymes (many by the cytochrome P450 oxidases in the liver) into other metabolites that may or may not have the same pharmacological effect as the parent drug.

The eye is unique in that many of the drugs for ocular therapy, particularly those designed to lower intraocular pressure, are formulated to be absorbed through the cornea and into other tissues of the eye, including the aqueous humor. The aqueous humor is a clear fluid that flows through the anterior segment of the eye that provides nutrients and drugs to other nonvascular eye tissues. After use, many eye drugs exit the eye, enter the blood stream, and are transported to the liver where they are metabolized. The drug metabolites or the drugs themselves are then excreted from the body either through urine or feces. However, it is important to

note that pathological conditions, genetic polymorphism, and drug–drug interaction may influence the pharmacokinetics of drugs.

Pharmacodynamics is the study of the biological effects of drugs and their mechanisms of action. Clinically, pharmacodynamics is the correlation between the dosage of a drug that is administered to a patient and the pharmacological response of the drug. It is important to note that there may not be a correlation between increases in dosages and pharmacological effects. One important reason is that the pharmacological effects of drugs are achieved through drug/receptor interactions. Drugs bind to their respective receptors, form a complex, and elicit their physiological effects. At base level, almost all receptors are available for the drugs to bind. As concentrations of drugs are increased, there are less available receptors with which to bind; therefore, the effects of the drugs may not change as drug/receptors reach saturation.

These receptor proteins can be located either on the plasma membrane, within the cytoplasm, or in the nucleus of a cell. The ability of a drug to bind to a receptor is governed by chemical/physical forces. Receptors can be classified as kinase receptors, ion channels, G protein-coupled receptors (those proteins coupled to G proteins), and intracellular receptors.

Two main terms used to describe the properties of drugs to receptors are potency and efficacy. Potency is related to dosage and efficacy is the ability of the drug to activate a receptor. As the potency of a drug decreases, the dose–response curve shifts to the right. An example is shown in Fig. 1 where drug A is more potent than drug B or C. Efficacy is a measure of the response of the drug and determined by a dose–response curve. For example, in Fig. 1, drug A and drug B have the same efficacy, but greater than drug C. ED_{50} is the concentration of drug where there is a half-maximal effect in vivo, EC_{50} is the concentration of drug where there is half-

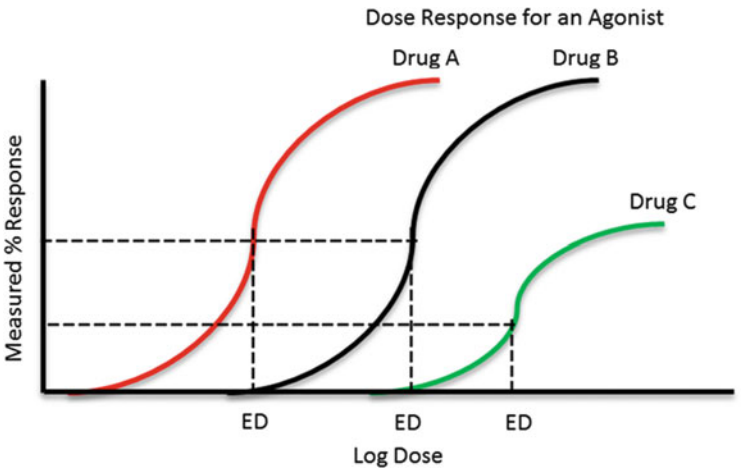

Fig. 1 Dose response curve of three different agonists visualizing drug potency and efficacy. Drug A and Drug B has the same efficacy but are more efficacious than Drug C. Drug A is more potent than Drug B and Drug C

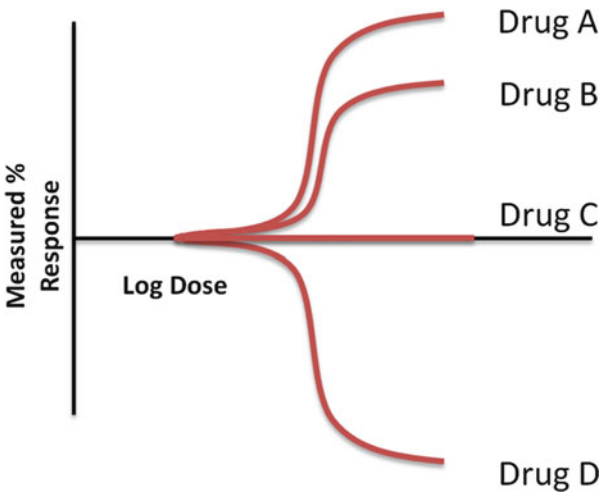

Fig. 2 Dose response curves of drug categorized into 4 groups by their response. Drug A is a full agonist which elicit a maximal response. A drug that does not give a full measured response is known as a partial agonist, which is depicted as Drug B. Drug C does not evoke a response and is an antagonist. Drug D produces a opposite response of Drug A and is categorized as an inverse agonist

maximal effect in vitro, and Kd is the concentration at which half-maximal binding of drugs to receptors occurs. In addition, depending on if studies for drugs and receptors are carried out in vitro or in vivo, the curves are called concentration–response curves or dose–response curves, respectively.

Drugs that produce a response (or have efficacy) can be broken down into 4 major categories: full agonist, partial agonist, antagonist, and inverse agonist. A full agonist will have a maximal response (drug A on Fig. 2), where as a partial agonist will have a less than maximal response (drug B on Fig. 2). A partial agonist in the presence of a full agonist can also be considered an antagonist. An inverse agonist is a phenomenon where a drug produces an opposite effect of that produced by an agonist (drug D on Fig. 2). Antagonist have no efficacy (drug C on Fig. 2) on a receptor and act either by blocking the direct binding site of an agonist (direct antagonist) or by binding to an indirect binding site, and changing the conformation of the receptor into its inactive state so that an agonist cannot bind to the receptor (Fig. 3). A direct antagonist can be overpowered by increasing the concentration of an agonist and would cause a shift of the dose–response curve to the right; on the other hand, an indirect antagonist in the presence of an agonist cannot be overcome by increasing the concentration of the agonist.

In terms of ophthalmology, one of the best examples to compare the potency of different drug classes to a particular pharmacological effect is the intraocular pressure (IOP)-lowering agents used to treat glaucoma. These agents include the first-line agent prostaglandins, beta-blockers (timolol), selective alpha-adrenergic agonist (brimonidine), and carbonic anhydrase inhibitors, like brinzolamide. Prostaglandins are considered first line for the treatment of glaucoma because they are the most potent topical agent that can be used to lower IOP, meaning that it takes less drug to get a desired therapeutic effect. If looking at the dose–response curves in Fig. 1, the prostaglandin agents would be drug A, while the other IOP lowering drugs would have their dose–response curves shifted to the right.

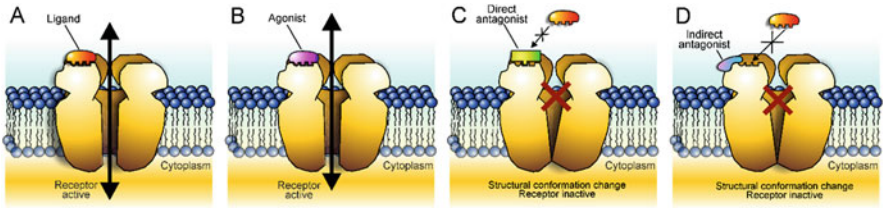

Fig. 3 Drug binding site of a receptor can cause conformational changes mediating receptor activity. When an endogenous ligand (A) or an agonist (B) binds to a receptor it produces a conformational change in the receptor opening up the receptor pore allowing the receptor to be in its active state. Ions are allowed to pass through the pore freely during the active state. A direct antagonist (C) can have affinity to the same binding site of endogenous ligands or agonists and thus competing against these ligands and agonists and preventing receptor activation. Indirect antagonist (D), bind to receptors in regions where ligands or agonist do not bind to. However, even though a ligand or agonist can bind, the receptor is inactive due to the indirect antagonist causing conformational changes inhibiting the receptor to open its pore channel

2 Receptor Profile

The eye is a complex sense organ that is composed of many unique types of tissues involved in light sensing and the visual transduction cascade. Within these tissues are cells that contain various classes of proteins that can act as receptors for pharmacological drugs to interact with and produce a measurable cellular/signaling response. Of the many families of receptors, there are four major receptor protein families that are able to transduce extracellular signals to intracellular responses, thus allowing these receptor families to be major drug targets for ocular therapeutics. In this section we will present an overview of these four receptor families: ligand-gated ion channels, G protein-coupled receptors, kinase-linked receptors, and nuclear receptors (Fig. 4).

2.1 Ligand-Gated Ion Channels

Ligand-gated ion channels (LGICs) (i.e., ionotropic receptors) are receptor protein channels embedded within the plasma membrane of neuronal cells. Following the binding of a neurotransmitter, these LGICs undergo a change in conformation and allow the influx of different ions such as Na^+, K^+, Ca^{2+}, or Cl^-, resulting in depolarization or hyperpolarization of the neuronal cell. The fast transmission of ions following the opening of LGICs allows these channels to be involved in fast synaptic transmission between cells in the neurons of the retina (Le Novere and Changeux 2001).

There are three superfamily classes of LGICs: pentameric, tetrameric, and trimeric receptors (Lemoine et al. 2012). The pentameric LGICs are named after

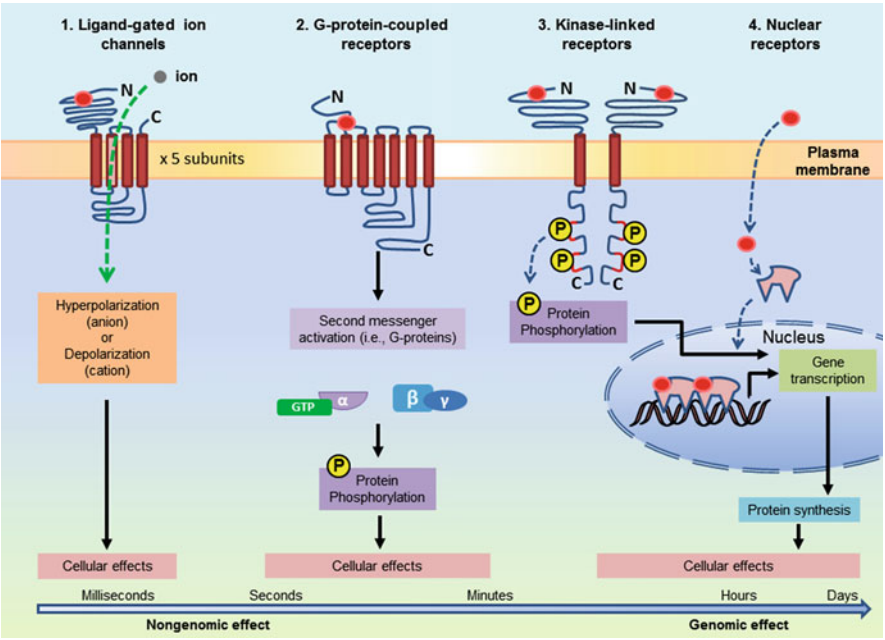

Fig. 4 A diagram of the four major receptor families, demonstrating their intracellular mechanism and the durations of their responses

five subunits that form homo- or heteromeric receptors. They are also known as Cys-loop receptors for the homologous extracellular loop that is flanked by two cysteine residues connected by a disulfide bridge. These receptors allow the movement of both cations and anions, which determines if the receptors are excitatory or inhibitory, respectively (Calimet et al. 2013). The excitatory LGICs consist of the serotonin, nicotinic acetylcholine, and zinc-activated ion channel, while the inhibitory receptors are the GABA and glycine receptors (daCosta and Baenziger 2013; Lemoine et al. 2012). The pentameric receptor's subunits have both the amino- and carboxyl-terminus on the extracellular region. These receptors include four transmembrane segments where the second membrane segment of each subunit associates with all five subunit second membranes to form a pore (Lemoine et al. 2012).

The second class of LGICs is the tetrameric receptors in which four homo or hetero subunits form the receptors. They are also known as the ionotropic glutamate receptors, which are comprised of the excitatory, nonselective cation permeable NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and kainate receptors (Mayer 2005). These receptor subunits contain an extracellular amino-terminal domain, an extracellular domain ligand-binding domain, a transmembrane domain (M1, M2, and M3 segment), and an intracellular carboxyl-terminal domain. Between M1 and M2 segment contains a loop that is partially reentrant to the plasma membrane, called the "p-loop" forming

an ion channel when other subunits are associated (Bruening-Wright et al. 2002; Traynelis et al. 2010).

The trimeric receptors are the third class of LGIC receptors where composed of the P2X receptors which is activated by ATP (Sanderson et al. 2014). These receptors are formed by three homo or hetero subunits having two transmembrane segments (M1 and M2). Both the amino and carboxyl-terminal domains of the subunits are located in the cytosol where there is an extracellular loop, rich in cysteine residues. These receptors are mainly permeable to cations but one type is permeable to chloride anions (Lemoine et al. 2012).

Cellular signal transduction through LGICs occurs when a specific extracellular ligand binds to the N-terminus of the receptor subunits. In the case of pentameric and trimeric receptors, the ligand-binding domain is between subunits, while in tetrameric receptors, the ligand-binding domain is within the core of each subunit (Connolly and Wafford 2004; Lemoine et al. 2012). The result of the ligand binding to the receptor causes a receptor conformational change therefore opening up the channel to allow ions to flow through. The speed of the cellular signal transduction occurs within milliseconds. There are no secondary messenger biochemical systems involved with LGICs. However, the influx of Ca^{2+} is important where it can act as a secondary messenger, activating many calcium-binding proteins, thus further amplifying intracellular signals and producing a signal transduction cascade which could influence gene expression and changes in cell physiology (Akopian and Witkovsky 2002; Pankratov and Lalo 2014).

2.2 G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) modulate, dampen, or enhance intracellular signals through the coupling of intracellular secondary messengers with members of the guanosine nucleotide-binding proteins called G proteins. These receptors are also known as metabotropic receptors or 7-transmembrane receptors (7TM receptors), and they are the largest group of the receptor families. Over 800 receptors belong to this family where they encode roughly 4% of the human genome (Katritch et al. 2013; Kobilka 2007). The large number of GPCRs makes them the biggest target for pharmaceutical therapeutics of the eye and nearly 50% of all drugs in the market target GPCRs (Xu and Xiao 2012).

There are five classes of GPCRs: rhodopsin family (710 members), adhesion family (24 members), frizzled/taste family (24 members), secretin family (15 members), and the glutamate family (15 members) (Katritch et al. 2013). Although being diverse, GPCRs generally are similar in structure where there are three main domains: an extracellular amino-terminal domain, seven alpha-helical (hydrophobic) transmembrane protein domain, and intracellular carboxyl-terminal domain. The transmembrane protein segments are the most conserved structure between the GPCRs, whereas the amino-terminus is the least conserved (Kobilka 2007).

Many different types of peptides/pharmacological agents can bind and act as ligands to GPCRs, ranging from photon-stimulating rhodopsin receptors in photoreceptor cells to the epinephrine-stimulating β2-adrenergic receptors in the nonpigmented ciliary epithelium (Crider and Sharif 2002; Okada et al. 2001; Orban et al. 2014). Many small organic molecules are able to bind to within the transmembrane segments; however, larger ligands such as protein normally bind to the extracellular amino-terminus or the extracellular loops between the transmembrane segments (Kobilka 2007). Binding of a ligand to the receptor results in a conformational change; this causes the receptor to act as guanine nucleotide exchange factor. The plasma membrane resident heterotrimeric G proteins (Gα, Gβ, Gγ) are recruited then to the GPCR, promoting an exchange of a GDP for a GTP on the alpha subunit resulting in the dissociation of the Gα subunit from the Gβγ subunit protein. The α subunit and the Gβγ subunit mediate other proteins as second messengers of GPCRs to transduce cellular signaling further downstream. The inherent GTPase activity of the Gα subunit causes hydrolysis of GTP to GDP, resulting in the termination of the G protein and reuniting of the α subunit to the Gβγ subunit (Johnston and Siderovski 2007).

GPCR cellular signaling is complex and widespread which occurs in a timescale of seconds to minutes. Activation of one ligand type can induce a multitude of effector systems. GPCRs can form homo- or hetero-oligomers causing activation of multiple effector systems simultaneously (Kobilka 2007). Additionally, G proteins are very diverse in which there are 21 different Gα subunits, at least 6 different Gβ subunits, and 12 Gγ subunits (Oldham and Hamm 2008; Smrcka 2008). Gα subunits are categorized in four major groups in which effector system they mediate (Simon et al. 1991).

1. G_s – Stimulates adenylyl cyclase and open Ca^{2+} channels and therefore increases cAMP
2. G_i – Opens K^+ channels and inhibits Ca^{2+} channels and adenylyl cyclase
3. G_q – Activates phospholipase C and regulates the inositol phosphate system; release of internal calcium store
4. $G_{12/13}$ – Activates Rho family GTPase signaling

The Gβγ subunit was believed to be a negative regulator of the Gα subunit; however, recently the Gβγ subunit was discovered to regulate other proteins such as ion channels, therefore increasing the complexity of GPCR cell signaling (Smrcka 2008).

2.3 Kinase-Linked Receptors

Kinase-linked receptors are large transmembrane receptors, containing a large amino terminal ligand-binding domain, an α-helical transmembrane segment, and a carboxyl catalytic domain. The effectors for these receptors range from wide variety of ligands such as growth factors, cytokines, insulin, and leptin. The binding

of a ligand triggers receptor dimerization, followed by the autophosphorylation of the catalytic carboxyl domain. This phosphorylation of the catalytic domains induces the recruitment of other second messenger proteins to associate to the kinase-linked receptor's catalytic domain to be phosphorylated and induce an amplified signaling cascade. Activation of kinase-linked receptor elicits signaling for gene transcription to facilitate synthesize of protein required for cell growth, proliferation, differentiation, and survival of the cell in a time period of hours (Hinck 2012; Lemmon and Schlessinger 2010; Wilks 1989).

There are four main classes of kinase-linked receptors bases on their phosphorylation moiety. There are the receptor tyrosine kinases (RTKs), the serine/threonine kinases (RSTKs), the cytokine receptors, and the guanylyl cyclase (GC)-linked enzyme. RTKs contains tyrosine kinase moiety, while RSTKs are a smaller class than RTKs with a serine/threonine kinase moiety. An example of RTKs being a target for ocular pharmacology are the vascular endothelin growth factor (VEGF) receptor, where overstimulation of the receptor signals for the formation of abnormal blood vessel growth observed in wet age-related macular degeneration (AMD) or diabetic macular edema (Davuluri et al. 2009; Triantafylla et al. 2014; Witmer et al. 2002). Cytokine receptors do not have kinase enzymatic activity itself and therefore need the use of intracellular tyrosine kinases such as JAK to phosphorylate the receptor itself as well as other proteins (Patel et al. 2013). GCs are similar to RTKs and are part of the G protein-signaling cascade where they synthesize cyclic GMP from GTP (Gileadi 2014). In the disease cone dystrophy, genetic mutations in guanylate cyclase 2D result in death of cone photoreceptor cells (Garcia-Hoyos et al. 2011).

2.4 Nuclear Receptors

Nuclear receptors (i.e., intracellular receptors or ligand-activated transcription factors) are important drug targets for their ability to recognize many gene regulatory sequences and regulate gene expression (Aranda and Pascual 2001). These receptors are not embedded in the plasma membrane of cells but found in the cytoplasm or within the nucleus. Ligands for nuclear receptors need to be lipid soluble to allow the ligands to pass through the plasma membrane freely where it binds to the receptor. The receptor then translocates to DNA, binding to a regulatory region near the promoter called a hormone response element, acting as a transcription factor. Co-activator and corepressor factors are then recruited mediating gene transcription. The activation of nuclear receptors is important for the regulation of metabolism, development, and homeostasis (Bain et al. 2007). These receptors can regulate many genes at once, for instance, glucocorticoid receptor has over 1000 genomic binding sites regulating the transcription of numerous genes (Polman et al. 2012). The timescale for gene transcription and protein synthesis mediated by nuclear receptors occurs between hours to days (Losel et al. 2003).

Nuclear receptors contain five regions: an amino terminal domain, a DNA-binding domain, a hinge region, a ligand-binding domain, and a carboxyl-terminal domain. The amino-terminal domain contains the activation function 1 (AF1) which regulates the binding and activity of the nuclear receptors to other transcription factors independently of a ligand. This region is the least conserved between different nuclear receptors. The DNA-binding domain contains a highly conserved DNA-binding domain containing two zinc fingers, which allow the receptor to recognized and bind to the hormone response element. The hinge region allows for dimerization of other nuclear receptors and transport of the nucleus. The ligand-binding domain contains activation function 2 (AF2) whose action is dependent on a bound ligand. Lastly, the carboxyl-terminal domain's function is the least understood where the domain is usually adjacent to the ligand-binding domain. (Aranda and Pascual 2001; Germain et al. 2006). Examples of nuclear receptors are glucocorticoid receptor, prostaglandin receptor (also GPCRs), retinoic acid receptor, and estrogen receptor.

3 Drug Receptor Targets as Modulators of Ocular Function

3.1 Ion Channel

An example of an ion channel as a target for therapy is the heteromultimeric epithelial sodium channel (ENaC), comprising of α β and γ subunits that form the functional channel. ENaC is a selective cation channel that is involved in the reabsorption of sodium ions in a variety of epithelial tissues including the lumen of the gut, lung airway epithelial cells, distal nephron, and the renal collecting duct and the plasma membrane of corneal epithelial cells and the conjunctiva, as well as sodium sensor in taste bud cells. ENaC is characterized by a conductance of 5 pS at physiological sodium concentrations and a half-saturation of ion conductance at 70 mM sodium and is selective for sodium over potassium. ENaC is constitutively active at the plasma membrane and changes between open and closed conformations with an average open probability of ~0.5.

ENaC is subjected to regulation by different factors including intracellular and extracellular sodium concentrations, serine proteases, and hormonal regulation and including the activation of second messenger systems.

Hormones control the expression of active ENaC at the plasma membrane by regulating the different steps of the biosynthetic pathway such as transcription, translation, or membrane trafficking.

In the eye ENaC contributes to the sodium homeostasis and maintenance of tear volume and ocular surface hydration (Krueger et al. 2012), making it an interesting drug target. Currently, a compound that inhibits ENaC, P-321, is in clinical trials for the treatment of dry eye (discussed in more detail below).

GPCRs currently targeted for therapies are the histamine receptors that are activated by histamine and alpha (α)- and beta (β)-adrenergic receptors, also called

adrenoceptors, that are activated by small-molecule catecholamines and prostaglandin receptors that are activated by the lipid-derived prostaglandins.

3.2 Alpha-Adrenergic Receptors

In the eye, studies of α-adrenergic receptor inhibition suggest that the α_2-adrenergic receptor regulates IOP (Mittag et al. 1985). Currently, there are three identified α_2-adrenergic receptor subunits expressed in ocular tissues as determined in studies in human eye using immunohistochemical, polymerase chain reaction, and dot-blot hybridization (Huang et al. 1995; Woldemussie et al. 2007). The α_{2A}-adrenergic receptor is localized in the anterior segment of the eye; in the nonpigmented ciliary epithelium, cornea, and conjunctival epithelia; and in the retina, including the somata of ganglion cell layer and inner nuclear layer somas. The α_{2B}-adrenergic receptor is located in the dendrites, axons, neurons, and glia. The α_{2C} immunostaining is present in pigmented ciliary epithelium, in corneal and conjunctival epithelial cells, and in the somata and inner segment of the photoreceptors.

The α-adrenergic receptors are G protein-coupled receptors; activation of these receptors results in inhibition of adenylate cyclase and decreases in cAMP. Activation of α_{2A}-adrenergic receptors in the aqueous humor-secreting ciliary processes results in decreased aqueous humor secretion via cAMP-mediated mechanisms (Jin et al. 1994; Ogidigben et al. 1994; Wang et al. 1993) and subsequent decreases in IOP.

3.3 Beta-Adrenergic Receptors

The β-adrenergic receptors are divided into three subtypes based on pharmacology and molecular cloning: β1-, β2-, and β3-adrenoceptors. Studies using fluorescent probes, autoradiography, and pharmacological tools have determined that the anterior eye segment is enriched with β-adrenergic receptors, particularly the β2 receptors (Elena et al. 1987; Jampel et al. 1987; Lahav et al. 1978; Nathanson 1980; Neufeld et al. 1978; van Alphen 1976; Waitzman and Woods 1971). The β-adrenergic receptors have been localized to the ciliary process epithelium, isolated ciliary process epithelial cells, blood vessel walls in the ciliary processes, episclera at the limbus, the iris in the region of the sphincter muscle, the trabecular meshwork, and the ciliary muscles. In fact, sympathetic nerve fibers innervate the ciliary processes (Ehinger 1964; ten Tusscher et al. 1989) and trabecular meshwork (Sears and Sherk 1963) suggesting endogenous regulation of aqueous humor dynamics by β-adrenergic stimulation.

Sympathetic stimulation and topically applied β-adrenergic agonist, epinephrine, decrease IOP, and paradoxically, β-adrenergic antagonist, timolol (which is effective clinically in treating glaucoma), also decreases IOP. Previously, it has been suggested that differences in sites of action of β-adrenergic agonists and

β-adrenergic antagonists might explain this complex action of adrenergic agents on IOP.

However, the regulation of and by the β-adrenergic receptors is complex. Traditionally, it is thought that binding of agonists to the receptors results in activation of adenylate cyclase, increased cAMP levels (Coca-Prados and Wax 1986), and activation of protein kinase A: activities thought to be short and transient. This may be because many GPCRs are desensitized through rapid phosphorylation by G protein-coupled receptor kinases within a period of less than 1 minute. These phosphorylation events may be more complex than previously thought, as phosphorylation by these kinases may result in switching of the coupling of the β2-adrenergic receptors to different G proteins to initiate different signaling outcomes (Daaka et al. 1997).

However, recent studies have demonstrated activation of β-adrenergic receptors, which resulted in elevated cAMP levels over 8 hours post agonist binding. These increases in cAMP levels are associated with extended physiological consequences. For example, activation of cardiac and neuronal β-adrenergic receptors via the sympathetic nerve fibers results in persistent contractile response in the heart during long periods of exercise and promotes long-term potentiation, necessary for learning and memory, respectively. These prolonged responses may be related to associations of the β-adrenergic receptors with scaffolding proteins, which may allow for complex interactions with the cAMP hydrolyzing enzyme, phosphodiesterase, that result in persistent β-adrenergic receptor signals (Fu et al. 2014).

3.4 Histamine Receptors

Histamine is a monoamine molecule synthesized from the amino acid L-histidine by histidine decarboxylase in certain cells, such as mast cells, basophils, enterochromaffin-like cells, and neurons. Histamine is ubiquitous and mediates inflammatory reactions via binding to histamine receptors. Currently, there are four identified histamine receptor subtypes: H_1, H_2, H_3, and H_4 receptors.

H_1 receptors are $G_{\alpha q}$ protein-coupled receptors that are expressed in many tissues in the body, including the eye, gastrointestinal tract, central nervous system, lungs, vascular smooth muscle cells, and endothelial cells. H_2 receptors are $G_{\alpha s}$ protein-coupled receptors expressed in the gastrointestinal tract, the central nervous system, smooth muscles, and endothelial cells. H_3 and H_4 receptors are $G_{\alpha i}$ protein-coupled receptors that are highly expressed in the central nervous system and blood cells, respectively. Of the four receptors, H_1 and H_2 are important drug targets with clinical therapeutic agents designed to block the effects mediated by these receptors; H_1 receptor blockers (ketotifen (pK_i 8.6), chlorpheniramine (pK_i 8.15), fexofenadine (pK_i 7.57), and desloratadine (pK_i 9.01)) are used as antihistamines to treat allergic reactions mediated by histamine, and H_2 receptor blockers (ranitidine and cimetidine) to treat gastric ulcers.

The H_1 receptors play a wide role in the pathological processes of allergy, including anaphylaxis, asthma, allergic rhinitis, atopic dermatitis, and

conjunctivitis. In addition to inflammatory allergenic effects of activation of H_1 receptors, it also triggers maturation of dendritic cells and modulates type 1 and type 2 T helper cells. Immunostaining, Western blots, and RT-PCR demonstrate H_1, H_2, and H_4 subtypes in mucosal biopsies from the human conjunctiva (Leonardi et al. 2011) and H_1 receptors in corneal endothelial cells (Srinivas et al. 2006). Pharmacological studies (Kirkegaard et al. 1982; Umemoto et al. 1987; Woodward et al. 1986), H_1 receptor knockout animals (Izushi et al. 2002), and RT-PCR (Leonardi et al. 2011) confirmed the involvement of the H_1 receptor in allergic inflammation associated with conjunctivitis.

Allergic conjunctivitis occurs when allergens enter the conjunctival stroma and bind to IgE on mast cells or basophils. The cross-linking of allergens and IgE on mass cells induces release of histamine from the mast cells and release of other allergic mediators including cytokines. In response to histamine release, blood vessels dilate and become permeable. Histamine binding to its receptors in the conjunctiva results in clinical manifestations of itching, swelling, and redness, which allow for several targets for drug intervention: mast cell stabilization, inhibition of histamine release, or inhibition of histamine receptors. Antagonism of the H_1 receptor is a targeted therapeutic approach for the treatment of these conditions.

3.5 Prostaglandin Receptors

The role of prostaglandins in regulating IOP was studied several years prior to identifying prostaglandin receptors in the eye. The effects of prostaglandin are biphasic: an initial short-term increase in IOP followed by a sustained decrease in IOP (Camras et al. 1977; Starr 1971).

The synthesis of prostaglandins from arachidonic acid is catalyzed by the enzymes cyclooxygenase (COX) and prostaglandin synthase. Prostaglandins are ubiquitous, and the types produced in a given cell are dependent on the expression profile of the prostaglandin synthetic enzymes in that particular cell. Prostaglandins are autocrine or juxtacrine modulators that have diverse pharmacological effects on the central nervous system and the cardiovascular, gastrointestinal, and visual systems. Additionally, prostaglandins have been associated with diseases such as cancer, inflammation, cardiovascular diseases, and hypertension. The use of non-steroidal anti-inflammatory drugs (NSAIDs) as inhibitors of cyclooxygenase in the clinical treatment of inflammatory diseases and the use of prostaglandin analogs in the treatment of glaucoma underscore the physiological importance of prostaglandins.

The biologically active prostaglandins (PG) are PGE_2, $PGF_{2\alpha}$, PGD_2, PGL_2 (prostacyclin), and TXA_2 (thromboxane) that interact with prostaglandin receptors EPs (1–4), FP, DP, IP, and TP, respectively. Quantitative autoradiography, in situ hybridization, immunohistochemistry, and RT-PCR confirmed EP and FP receptors in the ciliary epithelium, cornea, conjunctiva, iris sphincter muscle, longitudinal ciliary muscle, retinal ganglion cells, trabecular meshwork, sclera, Muller cells, and

optic nerves (Anthony et al. 2001; Davis and Sharif 1999; Matsuo and Cynader 1992; Ocklind et al. 1996; Schlotzer-Schrehardt et al. 2002), with differential distribution. The uveoscleral pathway through which aqueous humor exits the eye consists of the iris, ciliary muscles, supraciliary and suprachoroidal spaces, and the sclera, suggesting physiological regulation by endogenous prostaglandins. Clinically relevant targeting to the FP receptors result in IOP lowering.

FP receptors are Gq protein-coupled receptors; $PGF_{2\alpha}$ binding results in increased IP3/DAG and phosphorylation of myosin light-chain kinase (MLCK). In fact, the effects of latanoprost, a $PGF_{2\alpha}$ analog, are mediated via IP3. Studies have demonstrated that activation of the $PGF_{2\alpha}$/FP/IP3/MLCK system may result in contraction–relaxation of the iris sphincter muscle in the anterior segment of the eye that influences aqueous humor outflow and IOP lowering (Ansari et al. 2003).

3.6 Kinase-Linked Receptors

An example of a kinase-linked receptor that is clinically relevant as a therapeutic target is the vascular endothelial growth factor (VEGF) family of protein tyrosine kinase receptors; anti-VEGF therapy is currently used for the treatment of age-related macular degeneration. While there are five identified members of the VEGF family, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF), alternative splicing of corresponding mRNAs results in many isoforms of VEGF-A, VEGF-B, and PlGF. VEGF receptors include three protein tyrosine kinases, VEGFR-1, VEGFR-2, and VEGFR-3, and two nonenzymatic receptors, neuropilin-1 and neuropilin-2, localized to vascular endothelial cells. These specialized localization and distribution of VEGFRs allow for the selectivity and specificity of VEGF's actions. VEGFR-1 binds to VEGF-A, VEGF-B, and PIGF with high affinity, while VEGFR-2 binds with lower affinity to some isoforms of VEGF-A and higher affinity to VEGF-C and VEGF-D; however, binding results in different biological effects. VEGF plays a fundamental role in the process of neovascularization in normal physiological processes as VEGFR-1, VEGFR-2, and VEGFR-3 null mice failed to form organized blood vessels which resulted in death between embryonic days 7–9 (Dumont et al. 1998; Fong et al. 1995; Hiratsuka et al. 1998; Shalaby et al. 1995).

Studies in normal monkey eyes using RT-PCR and immunohistochemistry detected constitutively expressed mRNA and proteins of VEGF-A, respectively, particularly the VEGF121 and VEGF165 isoforms, in the conjunctiva, iris, retina, and choroid–retinal pigment epithelial layers. Within the retina, VEGF was expressed in the ganglion, inner nuclear layer (Stone et al. 1995), retinal pigment epithelial layer, and cone photoreceptors (Kim et al. 1999). VEGF receptors, VEGFR-1 and VEGFR-2 mRNA, were detected in the iris, the retina, and the choroid–retinal pigment epithelial layers (Kim et al. 1999; Wen et al. 1998).

There are alterations in the VEGF system in pathological states. Immunohistological studies in humans demonstrated increased VEGF expression in the retinal pigment epithelium and the outer nuclear layer in the maculae of

patients with age-related macular degeneration (Kliffen et al. 1997). Studies that demonstrated sustained release of VEGF resulted in retinal neovascularization and breakdown of the blood–retina barrier in rabbits and primates (Ozaki et al. 1997). Retinal branch vein occlusion in cats and primates resulted in increases of VEGF and activation of protein tyrosine phosphorylation and the tyrosine kinase pathway proteins, phospholipase C gamma and MAPK (Hayashi et al. 1997; Miller et al. 1994; Pierce et al. 1995).

4 Drug Interaction and Ocular Therapy

Of the many eye diseases and disorders, glaucoma, AMD, uveitis, and some corneal disorders are treated with medication. We will discuss the types of drug and receptor targets.

4.1 Cornea

The cornea is a clear, avascular structure that covers the front of the eye and serves as a protective agent and focuses the light to the retina. There are as many identified disorders and diseases that affect the cornea. Of these, we will briefly discuss the conditions that utilize drugs as therapeutic strategies.

4.1.1 Dry Eye

In individuals with dry eye, there is an imbalance in tear production, drainage, and absorption. This results in the eyes' inability to produce enough or good quality tears needed to keep the surface of the eye lubricated, help in wound healing, and protect against infection.

Currently, this condition is treated with artificial tears (lubricating drops) or compounds that allow the eye to produce tears, for example, cyclosporine (Kaswan et al. 1989; Yoshida et al. 1999), which is an immunomodulator. A cyclosporine ophthalmic emulsion (Restasis, www.restasis.com), the only FDA-approved drug to treat dry eye, works by causing the eye to produce tears. While the mechanism (s) of action is unknown, inhibition of calcineurin (a serene/threonine protein phosphatase) and subsequent modulation of T-cell activity may play a role (Gilger et al. 2013; Kapoor et al. 2010).

A novel ophthalmic solution called P-321 Ophthalmic Solution is in an FDA-approved phase 1 clinical trial (ClinicalTrials.Gov). P-321 is a potent epithelial sodium channel (ENaC) blocker (www.parion.com). Inhibition of ENaC prevents the absorption of tears by the cornea and conjunctiva which results in increased tear volume on the ocular surface (Hara et al. 2010).

P-321 was devised to be held on the cornea, with limited systemic distribution. The pharmacokinetic studies for P-321 demonstrate an IC_{50} of 1.9 ± 0.75 nM in human epithelial cells and a metabolic stability of P-321 in body blood products; which the drug is rapidly cleared from plasma. The effects of P-321/ENaC

modulation of tear volume are achieved within 30 min of drug administration and are maintained for at least 6 h.

4.1.2 Allergies

Allergies that cause the eye to itch and produce excess tears and burn are common in individuals particularly in warm, dry climates with high pollen count. Because histamines are mediators in allergic reactions, itching due to allergies is usually treated with antihistamines or histamine antagonists. Thus, blocking the actions of histamines prevents the itching associated with the allergic reactions.

In the eye, histamine binds to the histamine-1 (H1) receptor, a member of the GPCR family of receptors, and achieves its effects via activation of Gq and inositol phosphate increases and/or calcium mobilization.

Ketotifen fumarate ophthalmic solution (Zaditor drops; www.zaditor.com) is a noncompetitive histamine-1 (H1) receptor antagonist. Antihistamines block the release of histamine from the histamine-producing mast cells (Okayama et al. 1994) as well as block the binding of histamine to its receptors. Zaditor works by blocking both the H1 receptor and the release of histamine from mast cells.

Ketotifen fumarate pharmacokinetic profile was not obtained for topical use, but was obtained for intravenous, intranasal, oral, and rectal administrations. Bioavailability after oral administration was the lowest among the four routes, possibly due to the first-pass metabolism by the liver. No systemic effects were observed with topical application of the drug.

4.1.3 Conjunctivitis

Conjunctivitis or pink eye may occur because of bacteria or allergen. Bacterial conjunctivitis is treated with antibiotics (Azari and Barney 2013; Sheikh and Hurwitz 2001) such as azithromycin eye drops (www.azasite.com) (Cochereau et al. 2007), gatifloxacin ophthalmic solution (www.allergan.com), and levofloxacin. Allergic conjunctivitis is treated with bepotastine besilate ophthalmic solution (Bepreve, www.bausch.com) which is a selective H1 receptor antagonist (McCabe and McCabe 2012) and, like ketotifen fumarate, inhibits the release of histamine from mast cell.

4.1.4 Bacterial Infections

Bacterial keratitis is a common bacterial infection of the cornea caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (O'Brien 2003; Willcox 2011) which cause microbial contamination of contact lens. Activation of Toll-like receptors on corneal epithelial cells by *Pseudomonas aeruginosa* (Zhang et al. 2003) results in production of inflammatory mediators such as cytokines and chemokines (Sadikot et al. 2005). Aminoglycoside antibiotic solution such as gentamicin sulfate ophthalmic (www.bausch.com) is also used.

4.1.5 Viral Infections

Viral infections such as herpes zoster (shingles) which is caused by the varicella-zoster virus or ocular herpes caused by herpes simplex virus are treated with antiviral eye drops (Castela et al. 1994). Antiviral agents include both topical and oral medications. Examples of topical medications are idoxuridine ophthalmic (no longer used in the United States), ganciclovir ophthalmic gel (Colin 2007) (Zirgan, www.bausch.com), and trifluridine (Viroptic ophthalmic solutions). Ganciclovir is a DNA polymerase inhibitor and prevents viral replication by protein phosphorylation events (Littler et al. 1992). The mechanisms of action of trifluridine are not completely understood; however, it interferes with viral replication by blocking DNA transcription (Carmine et al. 1982; Suzuki et al. 2011).

4.2 Uvea

4.2.1 Uveitis

The uvea comprises the ciliary processes, choroid, and iris, and inflammation of these tissues is called uveitis. Uveitis can be secondary to other diseases including AIDs, tuberculosis, and sarcoidosis. The target tissues are the ciliary processes, choroid, and iris. To reduce the inflammation, corticosteroids, which may be anti-inflammatory or immunosuppressive agents, are used. Examples of steroid anti-inflammatory include prednisone and fluocinolone (Callanan et al. 2008; The Multicenter Uveitis Steroid Treatment Trial Research et al. 2011), and immunosuppressive agents include methotrexate, mycophenolate, azathioprine, and cyclosporine. In some cases, immune-specific biological response modifiers such as rituximab, abatacept, daclizumab, and the TNF-α inhibitors, infliximab and adalimumab, are also used (Larson et al. 2011; Nussenblatt et al. 1999; Smith et al. 2001). Prednisone, a mixture of glucocorticoid and mineralocorticoid, would likely bind to both intracellular glucocorticoid (GR) and mineralocorticoid receptor (MR) complexes and control gene transcription via direct and indirect mechanisms.

4.3 Retina/Anterior Eye Segment

4.3.1 Age-Related Macular Degeneration (AMD)

AMD is a leading cause of irreversible vision loss worldwide. AMD involves losses of cone photoreceptor cells in the macular region of the retina and results in blurred vision and eventually loss of central vision. Two types of AMD have been characterized, non-neovascular AMD (dry) and neovascular AMD (wet AMD). Aberrant VEGF expression has been associated with the pathophysiology of neovascular AMD (Kvanta et al. 1996) and results in abnormal growth of new blood vessels with structural defects that may lead to seepage of vascular contents and bleeding, thus leading to damages to the delicate macula. Of the two types of AMD, drug treatment options for neovascular AMD involve antivascular

endothelial growth factor (VEGF) therapy. This therapy is aimed at reducing the amounts of VEGF that is secreted in the eye, particularly the choroid layer of the eye.

The target tissues for anti-VEGF therapy are vascular endothelial cells. Examples of existing therapies include monoclonal antibody to VEGF (Krzystolik et al. 2002), such as bevacizumab (Avastin) and ranibizumab (Lucentis) (Avery et al. 2006; Rosenfeld et al. 2005, 2006); aptamers (small oligonucleotides that bind to VEGF), such as pegaptanib (Chakravarthy et al. 2006) (Macugen) that binds to and inhibits extracellular VEGF; and VEGF receptor proteins 1 and 2 that are fused to the Fc portion of IgG (Nguyen et al. 2006); this acts as a competitive receptor for endogenous VEGF and an example is aflibercept (Heier et al. 2012) (Eylea). Binding of these agents to VEGF decreases functional VEGF in the vascular tissues and halts the choroidal neovascularization and leakage from these immature blood vessels that cause damage to the retinal layers.

4.3.2 Glaucoma

While glaucoma is characterized as an optic neuropathy, current treatments available are aimed at decreasing IOP that is produced in the anterior segment of the eye. Maintenance of proper IOP depends on a unique balance between aqueous humor secretion by the ciliary processes and outflow through the trabecular meshwork and Schlemm's canal (pressure-dependent pathway) and the uveoscleral pathway (pressure-independent pathway). While some drugs have shown neuroprotective properties, no neuroprotective agent has gone past stage III clinical trials for the treatment of glaucoma. Studies involving human subjects (reviewed in van der Valk et al., 2009) demonstrated the efficacies of alpha-adrenergic agonists (alpha (α) agonist), beta-adrenergic antagonist (beta-blockers), carbonic anhydrase inhibitors, cholinergics (miotic), prostaglandin analogs, and combination therapies which are currently used in the treatment of ocular hypertension and glaucoma. Other IOP regulators and neuroprotective agents include the cannabinoids, Latrunculin A and B, rho kinase (ROCK) inhibitors, adenosine, nitric oxide, sigma-1 receptor agonists, and endothelin antagonists.

Examples of alpha (α)-adrenergic agonists are apraclonidine (Iopidine) which is only marketed in some countries and brimonidine tartrate (Alphagan P) and clonidine. Target tissues include the ciliary processes, the uveoscleral outflow pathway, and the retina (Toris et al. 1995; Wheeler et al. 2001). Activation of α_{2A}-adrenergic receptors in ciliary processes decreases aqueous humor secretion (Jin et al. 1994; Ogidigben et al. 1994; Wang et al. 1993). Although α-adrenergic receptors are localized in TM, studies fail to show regulation of conventional outflow facility. The retina contains the α_{2A}-adrenergic receptors (Wheeler et al. 2001), and brimonidine has been shown to be neuroprotective in glaucoma animal models (WoldeMussie et al. 2001) possibly by modulation of brain-derived neurotrophic factor (BDNF) (Gao et al. 2002).

Examples of beta (β)-adrenergic antagonists (β-blockers) are betaxolol HCl (a selective β_1 antagonist) and timolol (nonselective β_1- and β_2-adrenergic receptor antagonists), carteolol, metipranolol, and levobetaxolol. Target tissues are the

ciliary processes (Potter and Rowland 1978) which contain β_1- and β_2-adrenergic receptors and the retina (Elena et al. 1987; Ferrari-Dileo 1988). Activation of β-adrenergic antagonist decreases IOP by decreasing aqueous humor secretion in ciliary processes and decreasing the flow of aqueous humor. Both betaxolol HCl and timolol are neuroprotective in glaucoma animal models. While β-adrenergic antagonist is also involved in blood flow to the optic nerve head, it is not clear if its regulation of vascular tone is protective. Timolol, nonselective β_1- and β_2-adrenergic receptor antagonists, protects RGCs in rat glaucoma model. The signaling pathways by which β-blockers achieve their effects involve inhibition of adenylate cyclase and decreased cAMP in ciliary processes (Crider and Sharif 2002). In the retina inhibition of β-adrenergic receptors may involve regulation of calcium and sodium channels, NMDA receptors, and BDNF regulation.

Carbonic anhydrase inhibitors are brinzolamide (Azopt) and dorzolamide (Trusopt) and are used as eye drops. Methazolamide (Neptazane) and acetazolamide (Diamox) are used as oral medications (pills). Carbonic anhydrase inhibitors target the ciliary processes (Maren and Conroy 1993; Wistrand 1959) where they inhibit the enzyme carbonic anhydrase II, a major regulator of aqueous humor secretion.

The cholinergics (miotic), including pilocarpine and carbachol, target the ciliary muscles in the ciliary body. IOP is decreased by constriction of the ciliary muscles by the cholinergics. Ciliary muscle constrictions result in regulation of the trabecular meshwork and Schlemm's canal to increase outflow of aqueous humor.

Synthetic prostaglandin analogs include travoprost (Travatan), bimatoprost (Lumigan), tafluprost (Zioptan), and latanoprost (Xalatan) (reviewed in Toris et al., 2008). Like latanoprost, the others are PGF2α analogs and bind with high affinity to the FP receptor. Prostaglandins target the uveoscleral outflow pathway primarily to allow for increased pressure-independent outflow of aqueous humor. In some cases the trabecular meshwork and Schlemm's canal are regulated by prostaglandins. In experimental animal models, latanoprost has been shown to protect RGCs from death by antagonizing glutamate toxicity and inhibiting caspase 3 (Kanamori et al. 2009).

Combined therapies include brimonidine tartrate and timolol maleate, α agonist, and β-blocker; dorzolamide HCI and timolol maleate, carbonic anhydrase inhibitor, and β-blocker; and brinzolamide/brimonidine tartrate, carbonic anhydrase inhibitor, and α agonist.

4.4 Other Regulators of IOP

Cannabinoid CB1 receptors are localized in the trabecular meshwork and ciliary processes, while CB1 and CB2 mRNA are expressed in the retina. The cannabinoid receptors are G protein-coupled receptors and cannabinoids binding to its receptor decrease IOP by increasing aqueous humor outflow facility. In β-adrenergic knockout mice and CB(1)(-/-) mice, CB receptor agonist could not decrease IOP suggesting that CB1 receptor involvement in IOP regulation may be mediated by

β-adrenergics. In a rat model of glaucoma, cannabinoids have been shown to be protective.

Latrunculins A and B decrease IOP by increasing aqueous humor outflow facility by disrupting actin filaments in the trabecular meshwork and altering the cell's stiffness. Many of latrunculin's effects also involve the regulation of extracellular matrix proteins.

Rho kinase (ROCK) inhibitors target several tissues involved in IOP regulation and proper maintenance of vision, including the trabecular meshwork, the ciliary muscle, RGCs, and optic nerve head. ROCK decreases IOP by increasing aqueous humor outflow facility. In experimental animals, ROCK is also neuroprotective.

Adenosine receptors are expressed in the ciliary processes, trabecular meshwork, retina including Muller cells and RGCs, and the optic nerve head. Activation of adenosine receptors has been shown to lower IOP and, in experimental glaucoma, is involved in neuroprotection.

Studies have confirmed the involvement of renin–angiotensin system (RAS) in regulating IOP (reviewed in Vaajanen and Vapaatalo, 2011). Captopril, an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of systemic hypertension, reduced IOP in normal and glaucomatous individuals. Additionally, other hypertension-reducing agents, enalapril, ramipril, and fosinopril, reduced IOP in glaucoma animal models. More recent studies suggest that activation of the endogenous angiotensin-converting enzyme 2 (ACE2) and the Mas receptor decreased IOP in experimental model of glaucoma without changing systemic blood pressure (Foureaux et al. 2013).

Nitric oxide synthase, the synthetic enzyme of nitric oxide (NO), is expressed in the ciliary processes, trabecular meshwork, Schlemm's canal, retina, and optic nerve head. NO regulation of IOP is multifactorial. It decreases IOP by decreasing aqueous humor secretion, increasing aqueous humor outflow, and regulating vascular tone. NO could be neuroprotective or degenerative, depending on the concentrations in the tissue. In ciliary processes cholinergic stimulation regulates nitric oxide synthase activity (Ellis et al. 2001). In Schlemm's canal, shear stress causes increases in NO (Ashpole et al. 2014) which binds to soluble guanylate cyclase and activates the enzyme. In trabecular meshwork and Schlemm's canal, activation of soluble guanylate cyclase results in increased cGMP levels and activation of protein kinase G. The high conductance calcium-activated potassium channel is regulated by protein kinase G, and this regulation results in changes in cell volume and or cell contractility (Dismuke et al. 2008).

The sigma-1 receptor (σ-1r) is a 26kD transmembrane, non-opioid receptor that has been localized to the ciliary processes, retina, RGCs, and Muller cells. The σ-1r has been shown to be neuroprotective in RGCs both in vivo and in vitro (Mueller et al. 2014; Smith et al. 2008), by inhibiting overexpression of the apoptotic protein, Bax, and TNF-related apoptosis inducing ligand (TRAIL) and phosphorylation of JNK (Cantarella et al. 2007). The σ-1r regulation of IOP is dependent on the species; activation of the σ-1r results in decrease IOP in rabbits, although in σ-1r knockout mice there were no changes in IOP when compared to wild-type mice. The σ-1r is found on the endoplasmic reticulum (ER) and has the ability to form

complexes with the mitochondrion-associated ER membrane and/or translocate to interact with ionotropic channels located at the plasma membrane and appear to be involved in regulating the cells' ion channels (Mueller et al. 2013). In fact, many of the actions of σ-1r involve inhibition of voltage-gated channels or potentiation of ligand-gated channels.

Endothelin-1 is expressed in the iris, ciliary body, and retina. In pathological conditions there are increased levels of endothelin which are detrimental to the health of the tissue (Tezel et al. 1997). Endothelin receptors ET_A and ET_B are G protein-coupled receptors including $G_{\alpha s}$, $G_{\alpha i}$, and $G_{\alpha q}$ suggesting many different signaling pathways and multiple biological actions, whose actions are mediated by phospholipase C/inositol triphosphate and intracellular calcium. Above-normal endothelin levels result in an imbalance in the system resulting in sustained calcium influx, membrane depolarization, and eventual cell death. Inhibition of this system results in restoration of the tissues health (reviewed in Krishnamoorthy et al., 2008, and Prasanna et al., 2011).

References

Akopian A, Witkovsky P (2002) Calcium and retinal function. Mol Neurobiol 25(2):113–132

Ansari HR, Davis AM, Kaddour-Djebbar I, Abdel-Latif AA (2003) Effects of prostaglandin F2alpha and latanoprost on phosphoinositide turnover, myosin light chain phosphorylation and contraction in cat iris sphincter. J Ocul Pharmacol Ther 19(3):217–231

Anthony TL, Lindsey JD, Aihara M, Weinreb RN (2001) Detection of prostaglandin EP(1), EP(2), and FP receptor subtypes in human sclera. Invest Ophthalmol Vis Sci 42(13):3182–3186

Aranda A, Pascual A (2001) Nuclear hormone receptors and gene expression. Physiol Rev 81 (3):1269–1304

Ashpole NE, Overby DR, Ethier CR, Stamer WD (2014) Shear stress-triggered nitric oxide release from Schlemm's canal cells. Invest Ophthalmol Vis Sci 55(12):8067–8076

Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ (2006) Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 113 (3):363-372.e365.

Azari AA, Barney NP (2013) Conjunctivitis: a systematic review of diagnosis and treatment. JAMA 310(16):1721–1730

Bain DL, Heneghan AF, Connaghan-Jones KD, Miura MT (2007) Nuclear receptor structure: implications for function. Annu Rev Physiol 69:201–220

Bruening-Wright A, Schumacher MA, Adelman JP, Maylie J (2002) Localization of the activation gate for small conductance Ca2+-activated K+ channels. J Neurosci 22(15):6499–6506

Calimet N, Simoes M, Changeux JP, Karplus M, Taly A, Cecchini M (2013) A gating mechanism of pentameric ligand-gated ion channels. Proc Natl Acad Sci U S A 110(42):E3987–E3996

Callanan DG, Jaffe GJ, Martin DF, Pearson PA, Comstock TL (2008) Treatment of posterior uveitis with a fluocinolone acetonide implant: three-year clinical trial results. Arch Ophthalmol 126(9):1191–1201

Camras CB, Bito LZ, Eakins KE (1977) Reduction of intraocular pressure by prostaglandins applied topically to the eyes of conscious rabbits. Invest Ophthalmol Vis Sci 16 (12):1125–1134

Cantarella G, Bucolo C, Di Benedetto G, Pezzino S, Lempereur L, Calvagna R, Clementi S, Pavone P, Fiore L, Bernardini R (2007) Protective effects of the sigma agonist Pre-084 in the rat retina. Br J Ophthalmol 91(10):1382–1384

Carmine AA, Brogden RN, Heel RC, Speight TM, Avery GS (1982) Trifluridine: a review of its antiviral activity and therapeutic use in the topical treatment of viral eye infections. Drugs 23 (5):329–353

Castela N, Vermerie N, Chast F, Sauvageon-Martre H, Denis J, Godard V, Goldschmidt P, Pouliquen Y (1994) Ganciclovir ophthalmic gel in herpes simplex virus rabbit keratitis: intraocular penetration and efficacy. J Ocul Pharmacol 10(2):439–451

Chakravarthy U, Adamis AP, Cunningham ET Jr, Goldbaum M, Guyer DR, Katz B, Patel M (2006) Year 2 efficacy results of 2 randomized controlled clinical trials of pegaptanib for neovascular age-related macular degeneration. Ophthalmology 113(9):1508 e1501–e1525

Coca-Prados M, Wax MB (1986) Transformation of human ciliary epithelial cells by simian virus 40: induction of cell proliferation and retention of beta 2-adrenergic receptors. Proc Natl Acad Sci U S A 83(22):8754–8758

Cochereau I, Meddeb-Ouertani A, Khairallah M, Amraoui A, Zaghloul K, Pop M, Delval L, Pouliquen P, Tandon R, Garg P et al (2007) 3-day treatment with azithromycin 1.5% eye drops versus 7-day treatment with tobramycin 0.3% for purulent bacterial conjunctivitis: multicentre, randomised and controlled trial in adults and children. Br J Ophthalmol 91(4):465–469

Colin J (2007) Ganciclovir ophthalmic gel, 0.15%: a valuable tool for treating ocular herpes. Clin Ophthalmol (Auckland, NZ) 1(4):441–453

Connolly CN, Wafford KA (2004) The Cys-loop superfamily of ligand-gated ion channels: the impact of receptor structure on function. Biochem Soc Trans 32(Pt3):529–534

Crider JY, Sharif NA (2002) Adenylyl cyclase activity mediated by beta-adrenoceptors in immortalized human trabecular meshwork and non-pigmented ciliary epithelial cells. J Ocular Pharmacol Therapeut 18(3):221–230

Daaka Y, Luttrell LM, Lefkowitz RJ (1997) Switching of the coupling of the [beta]2-adrenergic receptor to different G proteins by protein kinase A. Nature 390(6655):88–91

daCosta CJ, Baenziger JE (2013) Gating of pentameric ligand-gated ion channels: structural insights and ambiguities. Structure 21(8):1271–1283

Davis TL, Sharif NA (1999) Quantitative autoradiographic visualization and pharmacology of FP-prostaglandin receptors in human eyes using the novel phosphor-imaging technology. J Ocul Pharmacol Ther 15(4):323–336

Davuluri G, Espina V, Petricoin EF 3rd, Ross M, Deng J, Liotta LA, Glaser BM (2009) Activated VEGF receptor shed into the vitreous in eyes with wet AMD: a new class of biomarkers in the vitreous with potential for predicting the treatment timing and monitoring response. Arch Ophthalmol 127(5):613–621

Dismuke WM, Mbadugha CC, Ellis DZ (2008) NO-induced regulation of human trabecular meshwork cell volume and aqueous humor outflow facility involve the BKCa ion channel. Am J Physiol Cell Physiol 294(6):C1378–C1386

Dumont DJ, Jussila L, Taipale J, Lymboussaki A, Mustonen T, Pajusola K, Breitman M, Alitalo K (1998) Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. Science 282 (5390):946–949

Ehinger B (1964) Distribution of adrenergic nerves to orbital structures. Acta Physiol Scand 62:291–292

Elena PP, Kosina-Boix M, Moulin G, Lapalus P (1987) Autoradiographic localization of beta-adrenergic receptors in rabbit eye. Invest Ophthalmol Vis Sci 28(8):1436–1441

Ellis DZ, Nathanson JA, Rabe J, Sweadner KJ (2001) Carbachol and nitric oxide inhibition of Na, K-ATPase activity in bovine ciliary processes. Invest Ophthalmol Vis Sci 42(11):2625–2631

Ferrari-Dileo G (1988) Beta 1 and beta 2 adrenergic binding sites in bovine retina and retinal blood vessels. Invest Ophthalmol Vis Sci 29(5):695–699

Fong GH, Rossant J, Gertsenstein M, Breitman ML (1995) Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 376(6535):66–70

Foureaux G, Nogueira JC, Nogueira BS, Fulgêncio GO, Menezes GB, Fernandes SOA, Cardoso VN, Fernandes RS, Oliveira GP, Franca JR et al (2013) Antiglaucomatous effects of the activation of intrinsic angiotensin-converting enzyme 2. Invest Ophthalmol Vis Sci 54 (6):4296–4306

Fu Q, Kim S, Soto D, De Arcangelis V, DiPilato L, Liu S, Xu B, Shi Q, Zhang J, Xiang YK (2014) A long lasting β1 adrenergic receptor stimulation of cAMP/protein kinase A (PKA) signal in cardiac myocytes. J Biol Chem 289(21):14771–14781

Gao H, Qiao X, Cantor LB, WuDunn D (2002) Up-regulation of brain-derived neurotrophic factor expression by brimonidine in rat retinal ganglion cells. Arch Ophthalmol 120(6):797–803

Garcia-Hoyos M, Auz-Alexandre CL, Almoguera B, Cantalapiedra D, Riveiro-Alvarez R, Lopez-Martinez MA, Gimenez A, Blanco-Kelly F, Avila-Fernandez A, Trujillo-Tiebas MJ et al (2011) Mutation analysis at codon 838 of the Guanylate Cyclase 2D gene in Spanish families with autosomal dominant cone, cone-rod, and macular dystrophies. Mol Vis 17:1103–1109

Germain P, Staels B, Dacquet C, Spedding M, Laudet V (2006) Overview of nomenclature of nuclear receptors. Pharmacol Rev 58(4):685–704

Gileadi O (2014) Structures of soluble guanylate cyclase: implications for regulatory mechanisms and drug development. Biochem Soc Trans 42(1):108–113

Gilger BC, Wilkie DA, Salmon JH, Peel MR (2013) A topical aqueous calcineurin inhibitor for the treatment of naturally occurring keratoconjunctivitis sicca in dogs. Vet Ophthalmol 16 (3):192–197

Hara S, Hazama A, Miyake M, Kojima T, Sasaki Y, Shimazaki J, Dogru M, Tsubota K (2010) The effect of topical amiloride eye drops on tear quantity in rabbits. Mol Vis 16:2279–2285

Hayashi A, Imai K, Kim HC, and de Juan E, Jr. (1997) Activation of protein tyrosine phosphorylation after retinal branch vein occlusion in cats. Invest Ophthalmol Vis Sci 38(2):372–380

Heier JS, Brown DM, Chong V, Korobelnik JF, Kaiser PK, Nguyen QD, Kirchhof B, Ho A, Ogura Y, Yancopoulos GD et al (2012) Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology 119(12):2537–2548

Hinck AP (2012) Structural studies of the TGF-betas and their receptors - insights into evolution of the TGF-beta superfamily. FEBS Lett 586(14):1860–1870

Hiratsuka S, Minowa O, Kuno J, Noda T, Shibuya M (1998) Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. Proc Natl Acad Sci U S A 95(16):9349–9354

Huang Y, Gil DW, Vanscheeuwijck P, Stamer WD, Regan JW (1995) Localization of alpha 2-adrenergic receptor subtypes in the anterior segment of the human eye with selective antibodies. Invest Ophthalmol Vis Sci 36(13):2729–2739

Izushi K, Nakahara H, Tai N, Mio M, Watanabe T, Kamei C (2002) The role of histamine H (1) receptors in late-phase reaction of allergic conjunctivitis. Eur J Pharmacol 440(1):79–82

Jampel HD, Lynch MG, Brown RH, Kuhar MJ, De Souza EB (1987) Beta-adrenergic receptors in human trabecular meshwork. Identification and autoradiographic localization. Invest Ophthalmol Vis Sci 28(5):772–779

Jin Y, Verstappen A, Yorio T (1994) Characterization of alpha 2-adrenoceptor binding sites in rabbit ciliary body membranes. Invest Ophthalmol Vis Sci 35(5):2500–2508

Johnston CA, Siderovski DP (2007) Receptor-mediated activation of heterotrimeric G-proteins: current structural insights. Mol Pharmacol 72(2):219–230

Kanamori A, Naka M, Fukuda M, Nakamura M, Negi A (2009) Latanoprost protects rat retinal ganglion cells from apoptosis in vitro and in vivo. Exp Eye Res 88(3):535–541

Kapoor KG, Mirza SN, Gonzales JA, Gibran SK (2010) Visual loss associated with tacrolimus: case report and review of the literature. Cutan Ocul Toxicol 29(2):137–139

Kaswan RL, Salisbury M, Ward DA (1989) Spontaneous canine keratoconjunctivitis sicca: a useful model for human keratoconjunctivitis sicca: treatment with cyclosporine eye drops. Arch Ophthalmol 107(8):1210–1216

Katritch V, Cherezov V, Stevens RC (2013) Structure-function of the G protein-coupled receptor superfamily. Annu Rev Pharmacol Toxicol 53:531–556
Kim I, Ryan AM, Rohan R, Amano S, Agular S, Miller JW, Adamis AP (1999) Constitutive expression of VEGF, VEGFR-1, and VEGFR-2 in normal eyes. Invest Ophthalmol Vis Sci 40 (9):2115–2121
Kirkegaard J, Secher C, Mygind N (1982) Effect of the H1 antihistamine chlorpheniramine maleate on histamine-induced symptoms in the human conjunctiva. Indirect evidence for nervous H1 receptors. Allergy 37(3):203–208
Kliffen M, Sharma HS, Mooy CM, Kerkvliet S, de Jong PT (1997) Increased expression of angiogenic growth factors in age-related maculopathy. Br J Ophthalmol 81(2):154–162
Kobilka BK (2007) G protein coupled receptor structure and activation. Biochim Biophys Acta 1768(4):794–807
Krishnamoorthy RR, Rao VR, Dauphin R, Prasanna G, Johnson C, Yorio T (2008) Role of the ETB receptor in retinal ganglion cell death in glaucoma. Can J Physiol Pharmacol 86 (6):380–393
Krueger B, Schlotzer-Schrehardt U, Haerteis S, Zenkel M, Chankiewitz VE, Amann KU, Kruse FE, Korbmacher C (2012) Four subunits (alphabetagammadelta) of the epithelial sodium channel (ENaC) are expressed in the human eye in various locations. Invest Ophthalmol Vis Sci 53(2):596–604
Krzystolik MG, Afshari MA, Adamis AP et al (2002) Prevention of experimental choroidal neovascularization with intravitreal anti–vascular endothelial growth factor antibody fragment. Arch Ophthalmol 120(3):338–346
Kvanta A, Algvere PV, Berglin L, Seregard S (1996) Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. Invest Ophthalmol Vis Sci 37(9):1929–1934
Lahav M, Melamed E, Dafna Z, Atlas D (1978) Localization of beta receptors in the anterior segment of the rat eye by a fluorescent analogue of propranolol. Invest Ophthalmol Vis Sci 17 (7):645–651
Larson T, Nussenblatt RB, Sen HN (2011) Emerging drugs for uveitis. Expert Opin Emerg Drugs 16(2):309–322
Le Novere N, Changeux JP (2001) LGICdb: the ligand-gated ion channel database. Nucleic Acids Res 29(1):294–295
Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141 (7):1117–1134
Lemoine D, Jiang R, Taly A, Chataigneau T, Specht A, Grutter T (2012) Ligand-gated ion channels: new insights into neurological disorders and ligand recognition. Chem Rev 112 (12):6285–6318
Leonardi A, Di Stefano A, Vicari C, Motterle L, Brun P (2011) Histamine H4 receptors in normal conjunctiva and in vernal keratoconjunctivitis. Allergy 66(10):1360–1366
Littler E, Stuart AD, Chee MS (1992) Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. Nature 358 (6382):160–162
Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, Wehling M (2003) Nongenomic steroid action: controversies, questions, and answers. Physiol Rev 83 (3):965–1016
Maren TH, Conroy CW (1993) A new class of carbonic anhydrase inhibitor. J Biol Chem 268 (35):26233–26239
Matsuo T, Cynader MS (1992) Localisation of prostaglandin F2 alpha and E2 binding sites in the human eye. Br J Ophthalmol 76(4):210–213
Mayer ML (2005) Glutamate receptor ion channels. Curr Opin Neurobiol 15(3):282–288
McCabe CF, McCabe SE (2012) Comparative efficacy of bepotastine besilate 1.5% ophthalmic solution versus olopatadine hydrochloride 0.2% ophthalmic solution evaluated by patient preference. Clin Ophthalmol (Auckland, NZ) 6:1731–1738

Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, Folkman J, Dvorak HF, Brown LF, Berse B et al (1994) Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. Am J Pathol 145(3):574–584

Mittag TW, Tormay A, Severin C, Podos SM (1985) Alpha-adrenergic antagonists: correlation of the effect on intraocular pressure and on α2-adrenergic receptor binding specificity in the rabbit eye. Exp Eye Res 40(4):591–599

Mueller BH 2nd, Park Y, Daudt DR 3rd, Ma HY, Akopova I, Stankowska DL, Clark AF, Yorio T (2013) Sigma-1 receptor stimulation attenuates calcium influx through activated L-type voltage gated calcium channels in purified retinal ganglion cells. Exp Eye Res 107:21–31

Mueller BH 2nd, Park Y, Ma HY, Dibas A, Ellis DZ, Clark AF, Yorio T (2014) Sigma-1 receptor stimulation protects retinal ganglion cells from ischemia-like insult through the activation of extracellular-signal-regulated kinases 1/2. Exp Eye Res 128:156–169

Nathanson JA (1980) Adrenergic regulation of intraocular pressure: identification of beta 2-adrenergic-stimulated adenylate cyclase in ciliary process epithelium. Proc Natl Acad Sci U S A 77 (12):7420–7424

Neufeld AH, Zawistowski KA, Page ED, Bromberg BB (1978) Influences on the density of beta-adrenergic receptors in the cornea and iris–ciliary body of the rabbit. Invest Ophthalmol Vis Sci 17(11):1069–1075

Nguyen QD, Shah SM, Hafiz G, Quinlan E, Sung J, Chu K, Cedarbaum JM, Campochiaro PA (2006) A phase I trial of an IV-administered vascular endothelial growth factor trap for treatment in patients with choroidal neovascularization due to age-related macular degeneration. Ophthalmology 113(9):1522 e1521–1522 e1514

Nussenblatt RB, Fortin E, Schiffman R, Rizzo L, Smith J, Van Veldhuisen P, Sran P, Yaffe A, Goldman CK, Waldmann TA et al (1999) Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial. Proc Natl Acad Sci U S A 96(13):7462–7466

O'Brien TP (2003) Management of bacterial keratitis: beyond exorcism towards consideration of organism and host factors. Eye 17(8):957–974

Ocklind A, Lake S, Wentzel P, Nister M, Stjernschantz J (1996) Localization of the prostaglandin F2 alpha receptor messenger RNA and protein in the cynomolgus monkey eye. Invest Ophthalmol Vis Sci 37(5):716–726

Ogidigben M, Chu TC, Potter DE (1994) Alpha-2 adrenoceptor mediated changes in aqueous dynamics: effect of pertussis toxin. Exp Eye Res 58(6):729–736

Okada T, Ernst OP, Palczewski K, Hofmann KP (2001) Activation of rhodopsin: new insights from structural and biochemical studies. Trends Biochem Sci 26(5):318–324

Okayama Y, Benyon RC, Lowman MA, Church MK (1994) In vitro effects of H1-antihistamines on histamine and PGD2 release from mast cells of human lung, tonsil, and skin. Allergy 49 (4):246–253

Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 9(1):60–71

Orban T, Jastrzebska B, Palczewski K (2014) Structural approaches to understanding retinal proteins needed for vision. Curr Opin Cell Biol 27:32–43

Ozaki H, Hayashi H, Vinores SA, Moromizato Y, Campochiaro PA, Oshima K (1997) Intravitreal sustained release of VEGF causes retinal neovascularization in rabbits and breakdown of the blood-retinal barrier in rabbits and primates. Exp Eye Res 64(4):505–517

Pankratov Y, Lalo U (2014) Calcium permeability of ligand-gated Ca^{2+} channels. Eur J Pharmacol 739:60–73

Patel AK, Syeda S, Hackam AS (2013) Signal transducer and activator of transcription 3 (STAT3) signaling in retinal pigment epithelium cells. Jak-Stat 2(4), e25434

Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE (1995) Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci U S A 92(3):905–909

Polman JA, Welten JE, Bosch DS, de Jonge RT, Balog J, van der Maarel SM, de Kloet ER, Datson NA (2012) A genome-wide signature of glucocorticoid receptor binding in neuronal PC12 cells. BMC Neurosci 13:118

Potter DE, Rowland JM (1978) Adrenergic drugs and intraocular pressure: Effects of selective β-adrenergic agonists. Exp Eye Res 27(6):615–625

Prasanna G, Krishnamoorthy R, Yorio T (2011) Endothelin, astrocytes and glaucoma. Exp Eye Res 93(2):170–177

Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90(3):262–267

Rosenfeld PJ, Moshfeghi AA, Puliafito CA (2005) Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration. Ophthalmic Surg Lasers Imaging 36(4):331–335

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY (2006) Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 355 (14):1419–1431

Sadikot RT, Blackwell TS, Christman JW, Prince AS (2005) Pathogen–host interactions in *Pseudomonas aeruginosa* pneumonia. Am J Respir Crit Care Med 171(11):1209–1223

Sanderson J, Dartt DA, Trinkaus-Randall V, Pintor J, Civan MM, Delamere NA, Fletcher EL, Salt TE, Grosche A, Mitchell CH (2014) Purines in the eye: recent evidence for the physiological and pathological role of purines in the RPE, retinal neurons, astrocytes, Muller cells, lens, trabecular meshwork, cornea and lacrimal gland. Exp Eye Res 127:270–279

Schlotzer-Schrehardt U, Zenkel M, Nusing RM (2002) Expression and localization of FP and EP prostanoid receptor subtypes in human ocular tissues. Invest Ophthalmol Vis Sci 43 (5):1475–1487

Sears ML, Sherk TE (1963) Supersensitivity of aqueous outflow resistance in rabbits after sympathetic denervation. Nature 197:387–388

Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC (1995) Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376 (6535):62–66

Sheikh A, Hurwitz B (2001) Topical antibiotics for acute bacterial conjunctivitis: a systematic review. Br J Gen Pract 51(467):473–477

Simon MI, Strathmann MP, Gautam N (1991) Diversity of G proteins in signal transduction. Science 252(5007):802–808

Smith JR, Levinson RD, Holland GN, Jabs DA, Robinson MR, Whitcup SM, Rosenbaum JT (2001) Differential efficacy of tumor necrosis factor inhibition in the management of inflammatory eye disease and associated rheumatic disease. Arthritis Care Res 45(3):252–257

Smith SB, Duplantier J, Dun Y, Mysona B, Roon P, Martin PM, Ganapathy V (2008) In vivo protection against retinal neurodegeneration by sigma receptor 1 ligand (+)-pentazocine. Invest Ophthalmol Vis Sci 49(9):4154–4161

Smrcka AV (2008) G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. Cell Mol Life Sci 65(14):2191–2214

Srinivas SP, Satpathy M, Guo Y, Anandan V (2006) Histamine-induced phosphorylation of the regulatory light chain of myosin II disrupts the barrier integrity of corneal endothelial cells. Invest Ophthalmol Vis Sci 47(9):4011–4018

Starr MS (1971) Further studies on the effect of prostaglandin on intraocular pressure in the rabbit. Exp Eye Res 11(2):170–177

Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, Keshet E (1995) Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. J Neurosci 15(7 Pt 1):4738–4747

Suzuki N, Emura T, Fukushima M (2011) Mode of action of trifluorothymidine (TFT) against DNA replication and repair enzymes. Int J Oncol 39(1):263–270

ten Tusscher MP, Klooster J, van der Want JJ, Lamers WP, Vrensen GF (1989) The allocation of nerve fibres to the anterior eye segment and peripheral ganglia of rats. II The sympathetic innervation. Brain Res 494(1):105–113

Tezel G, Kass MA, Kolker AE, Becker B, Wax MB (1997) Plasma and aqueous humor endothelin levels in primary open-angle glaucoma. J Glaucoma 6(2):83–89

The Multicenter Uveitis Steroid Treatment Trial Research G, Kempen JH, Altaweel MM, Holbrook JT, Jabs DA, Louis TA, Sugar EA, Thorne JE (2011) Randomized comparison of systemic anti-inflammatory therapy versus fluocinolone acetonide implant for intermediate, posterior and panuveitis: the multicenter uveitis steroid treatment trial. Ophthalmology 118 (10):1916–1926

Toris CB, Gleason ML, Camras CB, Yablonski ME (1995) Effects of brimonidine on aqueous humor dynamics in human eyes. Arch Ophthalmol 113(12):1514–1517

Toris CB, Gabelt BAT, Kaufman PL (2008) Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction. Surv Ophthalmol 53(Suppl 1):S107–S120

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62(3):405–496

Triantafylla M, Massa HF, Dardabounis D, Gatzioufas Z, Kozobolis V, Ioannakis K, Perente I, Panos GD (2014) Ranibizumab for the treatment of degenerative ocular conditions. Clin Ophthalmol 8:1187–1198

Umemoto M, Tanaka H, Miichi H, Hayashi S (1987) Histamine receptors on rat ocular surface. Ophthalmic Res 19(4):200–204

Vaajanen A, Vapaatalo H (2011) Local ocular renin–angiotensin system – a target for glaucoma therapy? Basic Clin Pharmacol Toxicol 109(4):217–224

van Alphen GW (1976) The adrenergic receptors of the intraocular muscles of the human eye. Invest Ophthalmol 15(6):502–505

van der Valk R, Webers CA, Lumley T, Hendrikse F, Prins MH, Schouten JS (2009) A network meta-analysis combined direct and indirect comparisons between glaucoma drugs to rank effectiveness in lowering intraocular pressure. J Clin Epidemiol 62(12):1279–1283

Waitzman MB, Woods WD (1971) Some characteristics of an adenyl cyclase preparation from rabbit ciliary process tissue. Exp Eye Res 12(1):99–111

Wang RF, Lee PY, Taniguchi T, Becker B, Podos SM, Serle JB, Mittag TW (1993) Effect of oxymetazoline on aqueous humor dynamics and ocular blood flow in monkeys and rabbits. Arch Ophthalmol 111(4):535–538

Wen Y, Edelman JL, Kang T, Zeng N, Sachs G (1998) Two functional forms of vascular endothelial growth factor receptor-2/Flk-1 mRNA are expressed in normal rat retina. J Biol Chem 273(4):2090–2097

Wheeler LA, Gil DW, WoldeMussie E (2001) Role of alpha-2 adrenergic receptors in neuroprotection and glaucoma. Surv Ophthalmol 45(Suppl 3):S290–S294

Wilks AF (1989) Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci U S A 86(5):1603–1607

Willcox MDP (2011) Review of resistance of ocular isolates of Pseudomonas aeruginosa and staphylococci from keratitis to ciprofloxacin, gentamicin and cephalosporins. Clin Exp Optom 94(2):161–168

Wistrand P (1959) The effect of carbonic anhydrase inhibitor on intra-ocular pressure with observations on the pharmacology of acetazolamide in the rabbit. Acta Pharmacol Toxicol (Copenh) 16:171–193

Witmer AN, Blaauwgeers HG, Weich HA, Alitalo K, Vrensen GF, Schlingemann RO (2002) Altered expression patterns of VEGF receptors in human diabetic retina and in experimental VEGF-induced retinopathy in monkey. Invest Ophthalmol Vis Sci 43(3):849–857

WoldeMussie E, Ruiz G, Wijono M, Wheeler LA (2001) Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. Invest Ophthalmol Vis Sci 42(12):2849–2855

Woldemussie E, Wijono M, Pow D (2007) Localization of alpha 2 receptors in ocular tissues. Vis Neurosci 24(5):745–756

Woodward DF, Ledgard SE, Nieves AL (1986) Conjunctival immediate hypersensitivity: re-evaluation of histamine involvement in the vasopermeability response. Invest Ophthalmol Vis Sci 27(1):57–63

Xu HE, Xiao RP (2012) A new era for GPCR research: structures, biology and drug discovery. Acta Pharmacol Sin 33(3):289–290

Yoshida A, Fujihara T, Nakata K (1999) Cyclosporin A Increases Tear Fluid Secretion via Release of Sensory Neurotransmitters and Muscarinic Pathway in Mice. Exp Eye Res 68(5):541–546

Zhang J, Xu K, Ambati B, Yu F-SX (2003) Toll-like Receptor 5-Mediated Corneal Epithelial Inflammatory Responses to Pseudomonas aeruginosa Flagellin. Invest Ophthalmol Vis Sci 44 (10):4247–4254

Ocular Pharmacokinetics

Chandrasekar Durairaj

Contents

Abstract

Although the fundamental concepts of pharmacokinetics remain the same, ocular pharmacokinetics has its own challenges due to the uniqueness of barrier properties posed by various ocular tissues and its growing complexity with different routes of ocular administration. A thorough understanding of the barrier nature will aid in tailoring a drug or its carrier's physicochemical properties to its advantage. In order to deliver the right payload of a drug at the target site, various approaches can be taken to leverage the pharmacokinetics that includes molecular design based on desirable physicochemical properties, formulation approaches, and alternative routes of administration. In this chapter, a brief overview of the barrier properties with respect to various routes of administration

C. Durairaj (✉)
Pfizer – Clinical Pharmacology, 10555 Science Center Drive, San Diego, CA 92121, USA
e-mail: Chandra.Durairaj@Pfizer.com

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_32

is presented along with the physicochemical properties that influence the pharmacokinetics of ocular drugs. Recent advances in ocular pharmacokinetics are discussed in addition to new perspectives in interpreting existing data.

Keywords
Anterior segment • Disposition • Intracameral • Intravitreal • Pharmacokinetics • Posterior segment • Topical

1 Introduction

The pharmacokinetic processes of absorption, distribution, metabolism, and elimination determine the time course of a drug in the body and the amount delivered to the site of action. An understanding of these interrelated processes is critical in deciding the dose and dosing frequency of a drug and thereby influences its efficacy and safety. The mode of delivering a drug including the route of administration and design of vehicle or carrier is dependent on its pharmacokinetic properties. The eye is a complex structure composed of several distinct tissues each with a specific function that poses numerous constraints to drug delivery. Due to the unique anatomy and physiology of eye, the pharmacokinetic process of a drug is affected by the ocular tissues and other barriers encountered in the administered route. Since a plethora of literature is available on the ocular structure and barriers to drug delivery, this chapter will provide a brief overview on this topic and will mainly focus on the recent advances in ocular pharmacokinetics with an emphasis on the influence of molecular and physicochemical properties that dictate the ocular fate of a drug. From a drug delivery perspective, anterior segment and posterior segment are the two major routes of ocular drug delivery which are entirely different and have unique properties (Fig. 1). The choice of administration route not only

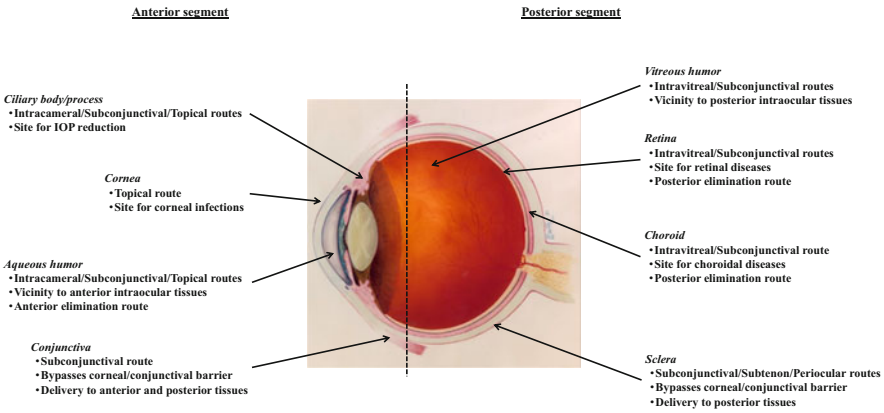

Fig. 1 Schematic representation of ocular delivery from a pharmacokinetic perspective. Adapted and modified from National Eye Institute, National Institute of Health (NIH)

depends on the target ocular tissue, but also on the barriers encountered in the route along with the physicochemical properties of the drug. Since the administration route has an impact on the ocular pharmacokinetics of a drug, this chapter provides an overview of the pharmacokinetic processes associated with the major routes of ocular drug delivery.

2 Ocular Pharmacokinetics of the Anterior Segment

The anterior segment of the eye constitutes cornea, conjunctiva, aqueous humor, lens, iris and ciliary body (ICB). The primary routes for drug delivery to the anterior segment include topical administration, subconjunctival, and intracameral injections. Pharmacokinetic processes involved with each of these major routes of administration are discussed below under each section.

2.1 Pharmacokinetics After Topical Administration

Topical administration is the most convenient route of drug delivery to the anterior segment of eye. Following topical instillation, majority of the administered drug is cleared rapidly from the ocular surface resulting in only 1–7% of the dose to reach the aqueous humor (Ghate and Edelhauser 2006). Precorneal clearance mechanisms including tear fluid turnover and blinking, selective permeability of the corneal epithelial barrier, and drug loss through nasolacrimal as well as systemic circulation attribute to the low bioavailability of drugs administered by this route.

2.1.1 Factors Affecting Absorption and Bioavailability

The critical factors that may affect the absorption process and alter the intraocular bioavailability of topical drops consist of physiological factors relevant to ocular tissues and molecular properties unique to drugs. A complete understanding of the interaction between these factors is essential to enhance the pharmacokinetic processes.

Loss of Drug from the Precorneal Surface

The tear volume in humans under normal condition is 7–9 μL with a turnover rate of 0.5–2.2 μL/min. Many commercially available eyedroppers deliver a typical volume of 25–56 μL to the precorneal tear film resulting in an increase in the tear volume. Under normal conditions, human palpebral fissure can hold 30 μL without overflowing. This abrupt increase in the volume due to topical instillation causes reflex blinking and rapid drainage from ocular surface. Majority of the applied medication is drained from the surface through the nasolacrimal duct and eventually cleared via systemic circulation.

Corneal Barriers of Drug Absorption

Cornea is the primary route for drug penetration to the anterior segments of eye following topical administration. The cornea is composed of epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium (Grass and Robinson 1988). The relative thickness of corneal epithelium, stroma, and endothelium are around 0.1:1:0.01. These three layers of cornea serve as substantial barriers for absorption. The epithelium is comprised of a basal layer of columnar cells, two to three layers of wing cells, and one or two outermost layers of squamous superficial cells. The superficial cells have tight intercellular junctions while the wing cells and basal cells consist of wider intercellular spaces. The tight junctions of the superficial corneal epithelial cells limit the absorption of hydrophilic drugs and favor transcellular permeation of lipophilic compounds. The paracellular route predominates for hydrophilic compounds of small molecular weight (<350 Da) (Ghate and Edelhauser 2006). Stroma is relatively a hydrophilic environment where drugs can diffuse through with minor resistance. Hydrophilic compounds with optimal molecular radius can easily diffuse through the stroma. Endothelium is a leaky barrier due to the large intercellular junctions between the monolayer of cells that partially resists penetration of lipophilic compounds.

Drug Properties Affecting Absorption

In case of topically administered drugs, absorption through cornea may occur via transcellular or paracellular pathways or by active transport. Drug properties that influence these processes such as lipophilicity and aqueous solubility play a key role in the penetration of drugs across cornea. Lipophilicity (Log*P*) in the range of 2–3 was found to be optimal for corneal permeation of steroids and β-blockers (Schoenwald and Huang 1983). An exploratory analysis of the apparent corneal permeability (P_{app}) values for more than 100 compounds indicated that corneal permeability is dependent on the distribution coefficient (Log*D* at experimental pH) (Prausnitz and Noonan 1998). As the dataset was mostly comprised of small molecules, no apparent dependency on molecular weight was observed. Further analysis of this permeability data with other molecular descriptors revealed potential correlation of corneal permeability with polar surface area (PSA) (Fig. 2) (unpublished data). PSA is the sum of surfaces of polar atoms, primarily oxygen, nitrogen, and their attached hydrogen atoms. PSA along with lipophilicity and molecular size influence the passive diffusion of molecules.

Due to the hydrophilic nature of corneal stroma, highly lipophilic compounds have limited permeability across this tissue. Stromal permeability data from a limited number of molecules ($N = 19$) indicated a strong dependence on its molecular weight and radius but no apparent relationship with any of the lipophilicity indicators (Log*P* or Log*D*) (Prausnitz and Noonan 1998). As indicated earlier, stroma is a thick, fibrous, and hydrophilic tissue where diffusion plays a major role in the transport of molecules. Thus stromal permeability is negatively correlated with molecular weight and radius, the parameters that affect the diffusion of a compound. Permeability of corneal endothelium shows a good correlation with both Log*D* and molecular radius indicating the role of both lipophilic and

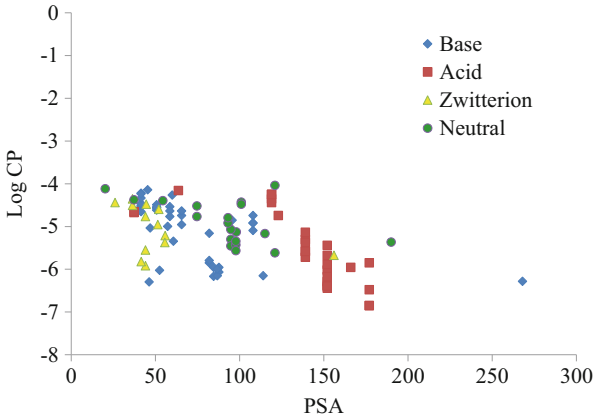

Fig. 2 Relationship between corneal permeability (CP) and polar surface area (PSA). Corneal permeability data comes from Prausnitz and Noonan (1998)

hydrophilic pathways. Similar to intact cornea, an increase in the corneal endothelial permeability was observed with a moderate increase in lipophilicity. However, the data was limited by the absence of highly lipophilic compounds to further investigate the barrier properties. Due to the presence of large intercellular junctions and leaky nature of the endothelial layer, as anticipated, strong correlation was observed with molecular radius. In general, taking into consideration the overall data, corneal epithelium serves as main barrier to transport of molecules across cornea. Small molecules with favorable lipophilicity readily cross corneal epithelium but stroma may provide a barrier to macromolecules.

Several formulation approaches are employed to overcome the absorption barriers and improve the ocular bioavailability. More information on these can be found in a recent review article (Ghate and Edelhauser 2006).

Non-corneal Routes of Absorption

Apart from the corneal route, topically administered drugs may be absorbed via non-corneal pathways that involve permeation across the conjunctiva and scleral layers. These routes play a major role in the penetration of drugs with poor corneal permeability that includes hydrophilic compounds and macromolecules (Ahmed and Patton 1985). Thus the drug properties determine the relative contribution of the non-corneal routes to absorption.

The conjunctiva is comprised of a stratified columnar epithelium and lamina propria. The superficial conjunctival epithelium has tight junctions with intercellular spaces wider than the corneal epithelium. Thus permeability of hydrophilic molecules is comparatively greater in conjunctiva. Further, large molecules such as inulin and FITC-dextran which are impermeable through cornea have limited permeability across conjunctiva. Based on the limited data available, no significant trend was observed between conjunctival permeability and lipophilicity while a possible dependency was observed with increasing molecular weight (Prausnitz and Noonan 1998). However, more data on large molecules is required to establish its role in conjunctival permeability.

2.1.2 Distribution of Drugs in the Anterior Segment of Eye

Topically administered drugs permeate across the cornea and enter the aqueous humor followed by distribution to the surrounding ocular tissues including iris–ciliary body, lens, choroid–retina, and vitreous (Ghate and Edelhauser 2006). Drugs that may exhibit non-corneal routes of absorption enter the uveal tract and vitreous without entering the aqueous humor. The rate and extent of drug distribution in the anterior segment is determined by a number of factors including permeability, diffusion in the aqueous humor, binding to proteins and surrounding ocular tissue components. Most of these factors are influenced by a drug's physicochemical properties including lipophilicity, solubility, and molecular weight. The apparent volume of distribution (V_d) of drugs can be measured by direct administration into the aqueous humor (intracameral). However, there is a paucity of data on the pharmacokinetics of drugs following intracameral injection. The V_d for few ophthalmic drugs administered by intracameral route is summarized along with key physicochemical properties in Table 1.

Based on the volume of aqueous humor in rabbits (0.3 mL), the V_d ranged from two- to tenfold larger than the aqueous humor volume. Although no clear trend was observed between V_d and molecular weight or Log*P*, drugs with higher protein binding had a lower V_d in the aqueous humor. Drugs that extensively bind to plasma proteins were known to exhibit a low V_d and can have a long plasma half-life. Flurbiprofen, a highly protein bound drug, has a longer elimination half-life in aqueous humor when compared to other moderate to weakly bound drugs (Table 1). For topically administered drugs, protein binding occurs first in the tear fluid which has a rapid turnaround time and as a result only the free unbound drug is available

Table 1 Volume of distribution (V_d) and elimination half-life ($t_{1/2}$) after intracameral injection of selected ophthalmic drugs in rabbits

Drug	V_d (mL)	$t_{1/2}$ (h)	MW	Log*P*	Protein binding (%)	Reference
Amikacin	2.67	0.58	586	−3.34	11	Mayers et al. (1991)
Chloramphenicol	3.33	0.69	323	1.02	50	Mayers et al. (1991)
Flurbiprofen	0.62	1.55	244	4.11	99	Tang-Liu et al. (1984)
Ibuprofen	0.53		206	3.72	90	Rao et al. (1992)
Levobunolol	1.65	0.67	291	2.86		Tang-Liu et al. (1987)
Moxifloxacin		2.2	434	0.01	50	Asena et al. (2013)
Pilocarpine	0.58		208	−0.095		Conrad and Robinson (1977)
Voriconazole[a]	0.65	0.4	349	0.93	58	Shen et al. (2009)

Drug physicochemical properties obtained from Durairaj et al. (2009) and Wishart et al. (2006)
[a]Pharmacokinetic parameters estimated by noncompartmental analysis using data from Shen et al. (2009)

for corneal absorption. More binding of the absorbed drug occurs in the cornea and aqueous humor. Protein content of the aqueous humor is different when compared to plasma. Concentration of proteins in the aqueous humor is approximately 200 times less than in plasma. However, these levels may increase in certain disease states that involve inflammatory conditions and subsequently result in increased binding of drugs. The effect of protein binding was investigated by adding increasing amounts of rabbit serum albumin to pilocarpine solution before topical administration (Mikkelson et al. 1973). The results indicated a 75- to 100-fold reduction in response (pupillary diameter) by the addition of 3% albumin indicating a decreased bioavailability as a result of protein binding. Nevertheless, more data comparing the pharmacokinetics of drugs in normal versus diseased state (e.g., inflammation, blood-aqueous barrier breakdown, etc.) is required to understand the effect of protein binding on the disposition of topically administered drugs.

From a therapeutic perspective, distribution of a drug to its target site is essential to achieve the desired efficacy. Although measurement of drug concentration in the aqueous humor provides an estimation of V_d, measuring drug levels in the surrounding ocular tissues is required to assess if the drug has reached the site of action. While pharmacokinetic studies with extensive tissue distribution data are scarce, few studies report the drug concentrations in key ocular tissues in addition to the aqueous humor. Given the number of animals required and the destructive nature of tissue sample collection, this is not uncommon in the ophthalmology field. Table 2 summarizes the AUC ratio of tissue:aqueous humor for few topical drugs of interest along with key physicochemical properties.

As expected, the relative exposure was higher in the cornea following topical instillation of drugs. The relative exposure in cornea was several folds higher for high molecular weight compounds (azithromycin and cyclosporin) when compared to other drugs. Also, the relative exposure of drugs in iris–ciliary body is higher than in aqueous humor. With the exception of lomefloxacin, for which data was available from infected rabbit eyes, the relative exposure in ICB decreased with increasing lipophilicity (Log*P*). Several explanations have been postulated to explain this higher exposure in ICB (Schoenwald 2003). Iris of rabbit eye is a porous and highly vascular tissue with majority of its surface area exposed to aqueous humor thereby allowing extensive distribution from aqueous humor. Further, an increased affinity/capacity for binding to melanin pigment in the iris could enhance the distribution to this tissue. Brimonidine, a drug well known to bind melanin, has higher relative exposure in ICB than in cornea (Table 2). Levobetaxolol, a cardioselective beta-adrenergic receptor blocking agent, has higher affinity to melanin with ICB exposure several folds higher than in aqueous humor.

An alternative explanation for the higher exposure in ICB could be due to potential contribution of non-corneal absorption routes via conjunctival/scleral pathways. Based on their physicochemical properties, certain drugs may be preferentially absorbed by conjunctiva and sclera to reach ICB without entering the aqueous humor. Chien et al. (1990) investigated the ocular absorption via corneal and conjunctival/scleral routes of clonidine, *p*-aminoclonidine, and AGN 190342

Table 2 Ocular tissue distribution of selected drugs after topical administration in rabbits

Drug	MW	Log*P*	Solubility (mg/mL)	Protein binding (%)	AUC ratio (Cornea/AH)	AUC ratio (ICB/AH)	Reference
Azithromycin[a]	749	3.03	0.514	51	784		Akpek et al. (2009)
Besifloxacin[a]	430	0.7	0.143	44	4.3		Proksch et al. (2009)
Bimatoprost[a]	416	3.41	0.0187	88		1.2	Shafiee et al. (2013)
Brimonidine[b]	442	1.27	0.154	29	4.4	116	Acheampong et al. (2002)
Cyclosporin[b]	1,203	3.35	0.00581	90	650	19	Acheampong et al. (1999)
Flunarizine	477	5.3	0.00168	99	3.5	2.9	Maltese and Bucolo (2003)
Gatifloxacin	375	−0.23	0.631	20	8.7		Durairaj et al. (2010)
Latanoprost[b]	433	4.16	0.0129	87	11.6	3.1	Sjoquist et al. (1998)
Levobetaxolol[a]	307			52		172	Review and Evaluation of Pharmacology and Toxicology Data (1999)
Lomefloxacin[c]	351	−0.39	0.106	10	5	18	Elena and Jauch (1997)

Drug physicochemical properties obtained from Durairaj et al. (2009) and Wishart et al. (2006)
[a]Pigmented animal
[b]Radiolabeled drug
[c]Infected eye

after drug perfusion in vivo. When drug was maintained over the conjunctiva over a period of time, the rank order of drug concentration in the anterior chamber tissues was conjunctiva > cornea > ciliary body > aqueous humor; whereas, when drug solution was in contact with cornea, the rank order for tissues was cornea > aqueous humor > ciliary body > conjunctiva. Besides, the conjunctival/scleral pathway was contributed as the predominant pathway for the least lipophilic (*p*-aminoclonidine) compound. Further experiments carried out using beta-blocking agents with varying lipophilicity, sucrose and inulin demonstrated that the outer layer of sclera provides less resistance to penetration of hydrophilic drugs when compared to cornea (Ahmed and Patton 1985). Moreover, the estimated permeability of conjunctival and scleral tissues was found to be 15–25 times higher than the cornea and was not affected by molecular size (Hamalainen et al. 1997).

2.1.3 Metabolism and Role of Transporters in Drug Disposition from the Anterior Segment

With the growing body of knowledge and evidence of its expression in various ocular tissues, drug metabolizing enzymes and transporters are gaining more attention from researchers to overcome the barriers for ocular drug delivery. Since there is an abundance of literature that provides a comprehensive overview of the distribution of these enzymes and their role in drug delivery (Attar et al. 2005; Zhang et al. 2008b), this chapter will focus only on key enzymes and transporters of clinical significance where in vivo evidence exists for their role in metabolism or drug–drug interaction (DDI).

Gene expression of aldehyde oxidase, an enzyme involved in oxidative metabolism, was detected in rabbit ocular tissues including ciliary body, iris, and cornea (Attar et al. 2005). Following a single topical administration of brimonidine, aldehyde oxidase mediated brimonidine metabolites were detected in the rabbit conjunctiva, cornea, and ICB (Acheampong et al. 2002). NADPH-dependent ketone reductase activity has been characterized in the corneal epithelium, ICB, conjunctiva, and the lens. After topical instillation, levobunolol undergoes reductive metabolism to dihydrolevobunolol in the corneal epithelium and ICB (Lee et al. 1988). Dihydrolevobunolol is an equally active metabolite with longer half-life than the parent drug and higher exposure in cornea, ICB, and aqueous humor.

Hydrolytic enzymes including esterases have been identified in several ocular tissues. Furthermore, there were recognized differences in their differential expression in various ocular tissues and among species. The major site for metabolism of dipivefrin, an anti-glaucoma agent, was identified as rabbit cornea although higher rates of metabolism were detected in ICB. Conversely, co-administration of an esterase inhibitor, echothiophate iodide in humans did not affect dipivefrin therapy indicating a lack of DDI (Mindel et al. 1981). The authors postulated that arylesterase could be responsible for the metabolism of dipivefrin (a phenol ester) which was not subject to inhibition by echothiophate iodide (a cholinesterase inhibitor). Besides, acetyl-, butyryl-, and carboxylesterases have been identified in the pigmented rabbit eye. Latanoprost, an isopropyl ester prodrug, is hydrolyzed by esterases in the cornea before reaching aqueous humor (Sjoquist and

Stjernschantz 2002). Aminopeptidase activity is also determined in various ocular tissues including corneal epithelium, ICB, conjunctiva, and aqueous humor of albino rabbits (Stratford and Lee 1985). Following topical administration of bimatoprost, a prostamide analog, bimatoprost acid levels were detected in aqueous humor and cornea indicating the involvement of aminopeptidase in the metabolism of bimatoprost (Shafiee et al. 2013).

Although the presence of various transporters in several ocular tissues has been characterized, the efflux pump transporter P-glycoprotein (P-gp) has been the most investigated. P-gp has been reported to exist in both corneal and conjunctival tissues (Dey et al. 2003). The corneal exposure of erythromycin, a lipophilic compound, was significantly increased in the presence of testosterone, a P-gp inhibitor indicating its significance in improving corneal bioavailability.

2.1.4 Elimination of Drugs from the Anterior Segment

Majority of the topically administered drug is lost through the nasolacrimal duct followed by systemic absorption. This portion of the drug is metabolized and eliminated by systemic pathways. Remaining drug undergoes intraocular absorption to reach the aqueous humor followed by distribution to surrounding ocular tissues. Elimination of drugs from the aqueous humor occurs by its turnover through the chamber angle and Sclemm's canal and by the venous blood flow of the anterior uvea (Schoenwald 2003). The turnover rate of aqueous humor in rabbit eye is 1.5% of the anterior chamber volume per minute which translates to a half-life of 46 min. Due to the rapid turnover rate of the aqueous humor, clearance of hydrophilic drugs will be faster than highly lipophilic drugs. This is further evident from the elimination $t_{1/2}$ of intracamerally administered drugs, where the $t_{1/2}$ ranged from 0.4 to 0.69 h for less lipophilic drugs while the $t_{1/2}$ of flurbiprofen (Log*P* 4.11) was 1.55 h (see Table 1). Table 3 summarizes the half-lives of few ophthalmic drugs of interest in various anterior ocular tissues following topical administration.

Table 3 Elimination half-lives of drugs in anterior ocular tissues following topical administration to rabbits

Drug	Log*P*	Cornea	Conjunctiva	AH	ICB	Tear	Reference
Azithromycin[a]	3.03	91	48	61		37	Akpek et al. (2009)
Besifloxacin[a]	0.7	6.1	6	12.1		6.1	Proksch et al. (2009)
Brimonidine[a]	1.27	13.3	9.17	3.06	17.3		Acheampong et al. (2002)
Gatifloxacin	−0.23	1.03	0.76	1.56			Durairaj et al. (2010)
Ketoconazole	4.3	0.72		0.32			Zhang et al. (2008a)
Loteprednol etabonate	2.2	3.75	4.26	2.31	3.04		Schopf et al. (2014)

[a]Pigmented animal

Drug physicochemical properties obtained from Wishart et al. (2006)

In case of topically administered highly lipophilic drugs (Log $P > 2$), elimination $t_{1/2}$ was longer in cornea when compared to other anterior chamber tissues. Corneal stroma, being hydrophilic, acts as a depot for highly lipophilic drugs and thereby slows its clearance from cornea.

2.2 Subconjunctival Pharmacokinetics for Drug Delivery to Anterior Segment

Although subconjunctival administration has been demonstrated to deliver drugs to the uvea, this route of administration is not familiar for the delivery of drugs to the anterior segment due to the morbidity of repeated subconjunctival injections. Since the corneal–conjunctival barrier is circumvented after subconjunctival injection, this route of administration is most beneficial for hydrophilic drugs. When gentamicin was administered by the subconjunctival route, sustained effective drug concentration was observed in patients undergoing cataract surgery (Baum and Barza 1983). Similar results were seen for subconjunctival vancomycin where substantially higher concentrations were observed in the aqueous humor in comparison to topical drops. More detail on this route of administration is provided in the later part of this chapter with relevance to posterior segment drug delivery (see Sect. 3.1.1).

2.3 Pharmacokinetics of Intracameral Administration

Direct injection of drug into the anterior chamber bypasses all the corneal and other external barriers to achieve higher drug levels in aqueous humor and surrounding ocular tissues. Results from a recent study indicate the benefits of intracameral injection of antibiotics. After analyzing a large number of cases in cataract surgery, a 22-fold drop in endophthalmitis was seen following the use of intracameral antibiotics (Shorstein et al. 2013). Due to the vicinity of target tissues involved in the regulation of intraocular pressure (IOP), direct injection of a drug or delivery system into the anterior chamber will have a beneficial effect when compared to topical delivery in the treatment of glaucoma. Utilizing this approach, recent research is focused on developing intracameral drug delivery systems mainly for sustained delivery of anti-glaucoma drugs. By administering a single intracameral implant made up of biodegradable polymeric delivery system containing travoprost in beagle dogs, sustained IOP lowering effect was maintained over 8 months with significantly lower aqueous humor concentration of travoprost in comparison to that of topical drops (Navratil et al. 2015). An intracameral implant containing 270 μg bimatoprost was designed to release the drug at a slower rate over 5 months (Hughes 2014). When injected into the anterior chamber of a beagle dog's eye, a sustained reduction in the IOP was observed for at least 5 months. A desired pharmacokinetic or pharmacodynamic profile can be achieved by controlling the release of drug from delivery system with the right proportion of its constituents. Data collected as part of screening various formulations can be collated to develop

an in vitro–in vivo correlation (IVIVC) model to optimize the formulation with desired drug release profile to achieve target concentration or pharmacologic response. These types of model can be developed in the absence of pharmacokinetic data as well by directly linking the in vitro dissolution profile to the pharmacodynamic response of interest.

2.4 Translational Pharmacokinetics for the Anterior Segment

Due to severe limitations in collecting serial pharmacokinetic samples from human eyes, the substantial reliance on data from animal studies is not uncommon in ocular drug development. Unlike the systemic drug development where allometric and physiologically based pharmacokinetic (PBPK) models are well recognized, interspecies scaling is not established in ocular pharmacokinetics. However, anatomical differences in the eye and physiological distinctions across various species can be integrated to augment the predictive capability of PKPD models in human. Utilizing this concept, a semi-mechanistic translational PKPD model was developed using pharmacokinetic and IOP data collected from rabbits and dogs to predict the IOP in human (Durairaj et al. 2014). The pharmacodynamic components of the model included diurnal variation in IOP and physiological parameters representing the turnover rate of aqueous humor in respective species (Fig. 3). Based on the assumption that differences in IOP across species can be attributed to their physiological differences in aqueous humor dynamics, IOP after drug treatment in human was simulated using the preclinical PKPD models. For human simulations, all model parameters representing PK and PD components were fixed and only the aqueous humor dynamics parameters (F_{in}, F_{us}, and C_{trab}) of animals were replaced with values for human. The model was able to predict the IOP in human based on the preclinical PKPD data with reasonable accuracy. Similar scaling approaches can be utilized with

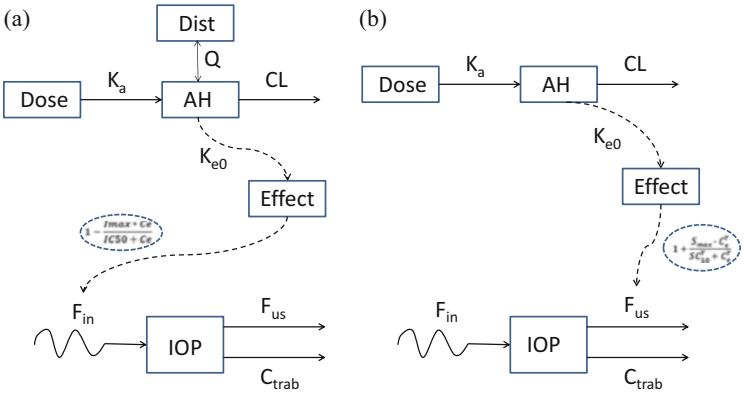

Fig. 3 A semi-mechanistic pharmacokinetic–pharmacodynamic model of (**a**) Brimonidine and (**b**) Latanoprost to predict IOP in patients. Reproduced with permission from (Durairaj et al. 2014)

an understanding of the mechanistic pathways and physiological differences to extend the predictability of PKPD models to human.

3 Ocular Pharmacokinetics of the Posterior Segment

From a pharmacokinetic perspective, drug delivery to the posterior segment tissues is unique and has its own challenges when compared to the anterior segment. Depending on the location of target site in the posterior segment of eye, various delivery routes can be employed to enhance the drug delivery. The most commonly employed routes include transscleral delivery and intravitreal injection.

3.1 Pharmacokinetics of Transscleral Delivery

Transscleral delivery typically comprises subconjunctival, retrobulbar, peribulbar, and sub-tenon injections. These routes are also called as periocular injections and are less invasive when compared with direct intravitreal injection. The relatively larger surface area of sclera and its unique properties in comparison with the cornea make it an attractive means of delivery to posterior segment tissues. Similar to corneal stroma, the permeability of sclera seems to have no dependence on the lipophilicity and a strong dependence on the molecular radius (Prausnitz and Noonan 1998). Based on in vitro experiments, large molecules such as dextran (40 kDa) and albumin (69 kDa) were shown to penetrate the sclera. However, the presence of scleral diseases and scleral thinning may pose additional menace to utilize this mode of drug delivery. In addition to these molecular properties, transscleral route is impeded by static, dynamic, and metabolic barriers (Shah et al. 2010). The static barriers comprise of sclera, Bruch's – choroid membrane, and retinal pigment epithelium (RPE) which have selective permeability to compounds of distinct physicochemical properties. The high blood and lymphatic flow rates in the conjunctiva and choroid constitute a dynamic barrier leading to higher clearance of drugs. Further, the presence of drug transporter proteins and efflux pumps pose another hurdle for delivery of drugs through this route. Enzymatic activity by cytochrome P450 and lysosomal enzymes may serve as metabolic barriers limiting the fraction available for absorption at this site.

3.1.1 Subconjunctival Pharmacokinetics for Drug Delivery to Posterior Segment

As mentioned earlier, the conjunctival–corneal barrier which is a substantial rate-limiting barrier for hydrophilic drugs is bypassed following subconjunctival injection as drug penetration occurs across sclera. The utility of this route for delivering various therapeutic agents to the posterior segment of the eye has been demonstrated in various studies (Shah et al. 2010). Barza et al. (1993) investigated the drug distribution after a single subconjunctival administration of four antibiotics in rabbits. Significant amount of drug levels were detected in the retina and vitreous humor following subconjunctival injection indicating drug penetration through the

scleral route to reach the posterior tissues. Subconjunctival administration of dexamethasone yielded substantially higher drug levels in the vitreous humor when compared to oral and peribulbar routes of administration (Weijtens et al. 2000). More direct evidence on the superiority of this route over topical delivery comes from a study comparing these routes for delivering bevacizumab, a humanized monoclonal antibody in rabbits (Nomoto et al. 2009). Bevacizumab exposure (both AUC and C_{max}) after subconjunctival injection was several folds higher in the retina/choroid and vitreous humor when compared to topical drops. As the drug has to permeate across retina/choroid to enter the vitreous, bevacizumab exposure (dose-normalized AUC) was higher in the retina/choroid than vitreous humor (645 vs. 45 ng. wk/g/mg) following subconjunctival administration. Moreover, due to the choroidal blood flow, substantial amount of drug is cleared into the systemic circulation before reaching vitreous humor. Kim et al. (2008) investigated the distribution and clearance of gadolinium-diethylenetriaminopentaacetic acid (Gd-DTPA) infused in the subconjunctival or intrascleral space of rabbits by means of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Results from this study indicated that subconjunctival infusion did not yield detectable levels of Gd-DTPA in the choroid/retina due to rapid clearance by conjunctival blood vessels and lymphatics in addition to the choroidal blood flow. Besides, the presence of RPE and the tight junctions between the endothelial cells of the retinal capillaries restrict the perfusion of drug into the retina and vitreous.

3.2 Pharmacokinetics of Intravitreal Administration

Injection of drug directly into the vitreous is an expedient way of delivering to the posterior tissues. Besides its invasive nature and other complications associated with intravitreal injections, this route of administration remains the pragmatic choice for drug delivery to the posterior segment diseases. Nomoto et al. (2009) compared the pharmacokinetics of bevacizumab after administration of repeated topical drops, a single subconjunctival injection or a single intravitreal injection in pigmented rabbits. Bevacizumab exposure (C_{max}) in the ICB and retina/choroid were 109,192.6 and 93,990 ng/g, respectively, after intravitreal injection, while the levels were 1,418.7 and 295.8 ng/g, respectively, following subconjunctival administration. Topical dosing of bevacizumab resulted in far less exposure than the other two administration routes. Similar trend was observed for AUC as well. The authors concluded that intravitreal injection was the most effective mode of delivering bevacizumab to intraocular tissues. Several articles demonstrate the enhanced delivery of small molecules and macromolecules to posterior tissues following intravitreal injection (Shah et al. 2010).

There are two major routes of elimination for drugs from the vitreous: anterior and posterior. In the anterior route, drugs diffuse across the vitreous to enter the posterior chamber followed by clearance through the aqueous humor turnover or uveal blood flow. In the posterior route, drugs permeate across the retina and eventually cleared by the choroidal blood flow. Due to the relatively large surface area, tissue partitioning, and involvement of active transport mechanisms,

Table 4 Ocular tissue distribution of selected ophthalmic drugs after a single intravitreal injection

Compound	MW	Log*P*	Elimination $t_{1/2}$ in vitreous (h)	AUClast (ng. h/g or μg. h/g)			Reference
				Vitreous humor	Aqueous humor	Retina/ choroid	
Brimonidine	442	1.37	4.9	21.5	0.43	132	Shen et al. (2014)
Dexamethasone	472	0.65	3.5	78.3	3.73	359	Zhang et al. (2009)
Foscarnet	126	−2.5	77	17,322		7,789	Lopez-Cortes et al. (2001)
Ganciclovir	255	−2.065	7.14	1,948		1,751	Lopez-Cortes et al. (2001)
Ketorolac tromethamine	376		2.28	612		833	Wang et al. (2012)
Melphalan	305	0.25	0.98	14.03	0.0956	19.52	Buitrago et al. (2010)
Moxifloxacin	434	0.01	9.4	486	33		Iyer et al. (2005, 2006)
Ranibizumab	48,350		63.12	689	231	221	Gaudreault et al. (2005)
Rituximab	143,860		111.6	2,096.6	315.7		Kim et al. (2006)
Topotecan	421	1.84		26.62	1.35		Buitrago et al. (2010)
Vancomycin	1,449	−1.44	33	18,488	2,607		Coco et al. (1998)
Voriconazole	349	0.93	2.6	77.8	0.6475		Shen et al. (2007)

Drug physicochemical properties obtained from Durairaj et al. (2009) and Wishart et al. (2006)

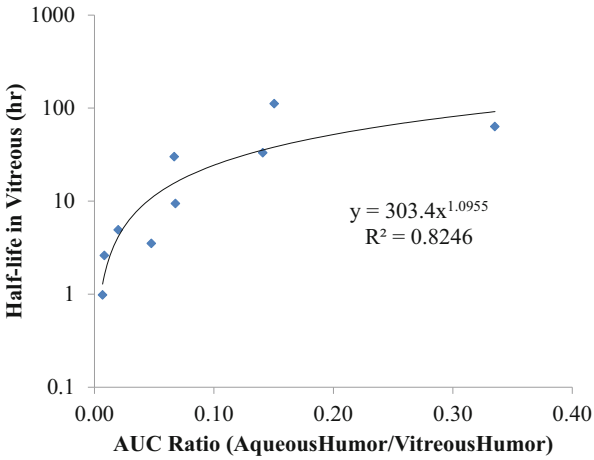

Fig. 4 Relationship between elimination half-life in vitreous and the ratio of AUC (aqueous humor/vitreous humor) for intravitreally injected drugs

molecules eliminated by the posterior (retinal) pathway have typically short half-life in the vitreous. Thus, the molecular and physicochemical properties of a drug play a major role in determining the primary elimination route from the vitreous humor. Table 4 shows the drug exposure (AUC) in vitreous, aqueous, and retina/choroid tissues after a single intravitreal injection of few ophthalmic drugs of interest in rabbit or monkey eyes.

As evident from Fig. 4, an exponential relationship exists between the half-life of drugs in the vitreous and the ratio of AUC (aqueous humor/vitreous humor). The elimination half-life in vitreous is shorter for drugs with lower partitioning ratio from vitreous to aqueous humor. In other words, drugs that are predominantly eliminated from the vitreous through the anterior pathway (aqueous humor turnover) have longer half-lives in the vitreous. Although a clear relationship cannot be elucidated with the limited data, the overall trend indicates a decrease in the vitreal half-life with increasing lipophilicity consistent with the expectation (see Table 4). As indicated earlier, adequate lipophilicity is required to penetrate the tight junctions of RPE barrier which results in large molecular weight and low lipophilic compounds to have prolonged half-life in the vitreous.

Maurice and Mishima (1984) demonstrated the relationship between molecular weight and aqueous/vitreous ratio indicating that primary route of elimination for high molecular weight compounds is by way of the anterior chamber. Dias and Mitra (2000) showed an inverse relationship between the molecular weight and vitreous elimination rate constant for high molecular weight FITC-dextrans. Using computer generated concentration contours, Maurice (2001) demonstrated that high molecular weight compounds exhibit prolonged half-life in vitreous. Figure 5 shows the relationship between Log*P* and the AUC ratio of retina-choroid/vitreous humor. Although, only limited data was available, the dependence on lipophilicity is clearly evident for partitioning into the retina/choroid which is in accordance with the previous reports. Similar results were reported by Liu et al. (1998) for a

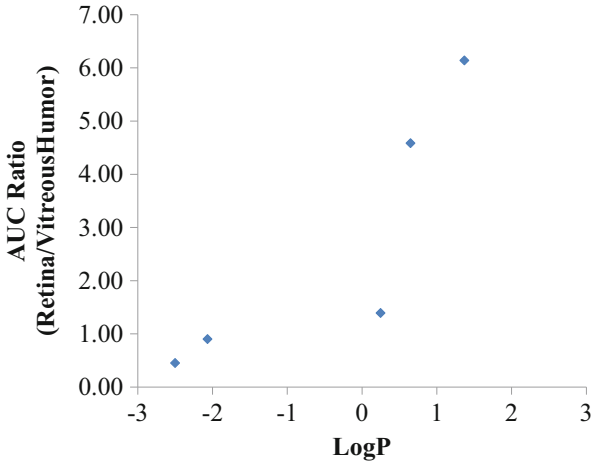

Fig. 5 Dependence on lipophilicity (LogP) for partitioning into retina from vitreous after intravitreal injection

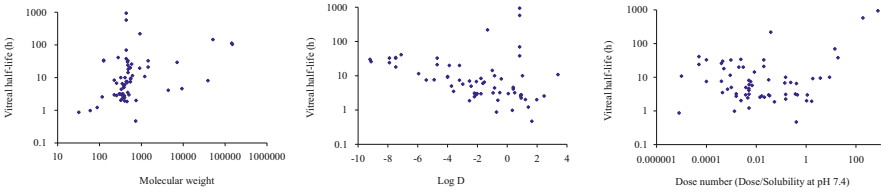

Fig. 6 Correlation between vitreal half-life and key physicochemical properties. Reproduced with permission from Durairaj et al. (2009)

small group of structurally similar antibiotics where an excellent correlation between lipophilicity and vitreous elimination was observed.

A comprehensive relationship was developed using a diverse set of compounds to establish the relationship between physicochemical properties and half-life of drugs in the vitreous (Durairaj et al. 2009). A multiple linear regression analysis was conducted to identify the physicochemical properties that are predictors of half-life of a drug in the vitreous. The correlation model developed indicated that molecular weight, lipophilicity (Log*P* or Log*D*), and dose number (dose/solubility at pH 7.4) are the significant physicochemical properties that impact the half-life of molecules in the vitreous. The general model developed using the entire dataset (Log $t_{1/2} = -0.178 + 0.267$ Log MW – 0.093 Log $D + 0.003$ Dose/Solubility$_{7.4} + 0.153$ PF) predicted the half-life of drugs in vitreous with good accuracy ($R^2 = 0.725$). Figure 6 shows the relationship between vitreal half-life and the key physicochemical properties identified in the regression model as significant contributors.

Dose number is a derived variable that includes the dose administered and its solubility at pH 7.4. When the injected dose exceeds its solubility in the vitreous, a depot or suspension is formed thereby releasing the drug in a sustained manner. As evident from the Fig. 6, when the dose injected exceeds the solubility limit (Dose/Solubility at pH 7.4 > 1), a steep increase in the apparent elimination half-life of drug was observed due to the slow release of the drug from the suspension or depot. Thus, including the dose and solubility improved the prediction for suspension formulations as well. Besides this general model, a number of submodels were also developed for various subsets depending on the dosage form administered (solution vs. suspension), animal model (albino vs. pigmented), ionization state (acids, base, neutral, and zwitterions), and molecule size (small vs. macromolecules). The models developed for these subsets provided insight into the key molecular properties that are unique for each of those classes. For instance, molecular weight was the major determinant of the half-life for macromolecules while lipophilicity was the main predictor for the acidic, basic, and zwitterionic compounds.

Kidron et al. (2012) used a narrower molecular weight range of compounds (<1,500 Da) to develop a model to predict the intravitreal half-life using 33 physicochemical descriptors relating to lipophilicity, hydrogen bonding, and mass. The final model for whole dataset included Log*D* at pH 7.4 and the total number of hydrogen bonds as predictors of half-life ($\log t_{1/2}$, mixed $= -0.046 - 0.051$ (log*D*7.4) + 0.640 (Log*H*tot). Since compounds with molecular weight <1,500 Da were only included in the dataset, the final model did not include MW as a key descriptor. However, the model developed had a good predictive capability with Q^2 of 0.64 using the training set and 0.69 using the test set.

3.3 Effect of Pigmentation on Ocular Pharmacokinetics

In the eye, melanin is primarily distributed in ICB, choroid, and RPE. Moreover, regional differences in the distribution of melanin within RPE have been reported in human eyes (Schmidt and Peisch 1986) and in animals (Durairaj et al. 2012). Despite the route of administration, ocular drugs encounter these pigmented tissues during their pharmacokinetic life cycle as part of distribution or elimination process. Akin to protein binding, binding of drugs to melanin has raised interest in investigating its role in the disposition of drugs from eye. Few studies have investigated the pharmacokinetics of intravitreally injected drugs in pigmented animals. Table 5 summarizes the vitreal half-lives of compounds reported in both albino and pigmented rabbits after intravitreal injection. For at least more than half of the compounds, vitreal half-life is longer in pigmented than in albino rabbits (Table 5). However, with this limited set of data no clear trend can be established with any physicochemical properties.

Melanin is a polyanionic polymer comprised of repeating units of 5,6-dihydroxy indole-2-carboxylic acid and 5,6-dihydroxy indole (Nofsinger et al. 2000). Identification of key molecular properties that influence binding to melanin is more complex and depends on the nature of interaction (reversible vs. irreversible),

Table 5 Half-life of drugs in vitreous after intravitreal injection in albino and pigmented rabbits

Compound	Half-life in vitreous (h)	
	Albino rabbits	Pigmented rabbits
Acyclovir	2.98	8.36
Aztreonam	8.3	7.5
Carbenicillin	3.5	5
Cefazolin	1.86	7
Ceftazidime	7.4	20
Ceftriaxone	6.75	9.1
Foscarnet	34	77
Ganciclovir	2.62, 7.1	6.98, 8.66
Grepafloxacin	3	2.9
Vancomycin	21	32.67, 62.34

Data obtained from Durairaj et al. (2009)

chemical groups involved in binding, ionization status at the given pH, etc. Besides, the extent to which melanin binding can alter the pharmacokinetics of a drug is also dependent on the predominant route of elimination for that compound from the vitreous.

3.4 Influence of Disease State on Ocular Pharmacokinetics

The effect of disease state on the pharmacokinetics of ocular drugs is one of the less explored areas in ocular drug delivery. Barza et al. (1993) investigated the pharmacokinetics of cephalosporins after subconjunctival and intravitreal injections in the normal and infected eyes of rabbits. Repeated subconjunctival injections in the infected eyes resulted in two- to ninefold higher drug concentration in the vitreous when compared to a single subconjunctival injection in normal eyes. However, these higher levels in the infected eyes were probably related to repeated dosing rather than inflammation. The half-life of ceftizoxime and ceftriaxone were longer in the infected eyes than in normal eyes after intravitreal injection. This is presumably due to the ocular inflammation that generally causes damage to the transport pump and thereby prolonging the half-life of drugs that are eliminated by the posterior (retinal) route. After intravitreal injection in rabbits, the vitreal half-life of ketorolac in normal eyes was 2.28 h (Wang et al. 2012) while the half-life was 4.27 h (Baranano et al. 2009) in eyes with ocular inflammation. Similar results were reported by other authors where the vitreal half-life of ceftriaxone (Jay et al. 1984) and cefazolin (Ficker et al. 1990) was longer in the aphakic eye when compared to phakic eyes. Conversely, a decrease in the vitreal half-life of vancomycin was reported following intravitreal injection in infected rabbit eyes (Coco et al. 1998). The vitreal half-life decreased from 62.3 h in normal eyes to 13.6 h in infected eyes. This increased clearance was attributed to the increased permeability due to the disruption of blood–retinal barrier (BRB).

Cheruvu et al. (2009) investigated the effect of diabetes on transscleral delivery of celecoxib in rats. Following induction of diabetes in albino and pigmented rats, a breakdown in the BRB was observed with 2.4- to 3.5-fold higher leakage than in controls. When a single periocular injection of celecoxib was administered to both the rats with BRB breakdown, celecoxib exposure was 1.5- and 2-folds higher in the retina and vitreous humor of treated eyes as a result of the disruption of the BRB.

Shen et al. (2014) compared the ocular pharmacokinetics of brimonidine and dexamethasone after a single intravitreal injection in rabbits and monkeys with BRB breakdown. In case of rabbits, dexamethasone exposure (AUC) in aqueous humor, retina, and choroid was lower in disease animals than in normal animals. Similar trend was observed for brimonidine as well. In contrary, the central retina/choroid region where choroidal neovascularization (CNV) was established by laser lesions was the only ocular tissue in monkeys with consistent lower drug exposure. The AUC for brimonidine and dexamethasone was significantly higher by 59% and 23%, respectively, in normal animals when compared to CNV monkeys ($P < 0.05$). In addition to the anatomical and physiological differences, different induction methods were used in these species to disrupt the BRB that could have contributed to this difference. Besides, these results supported the enhanced clearance in animals with BRB breakdown thereby resulting in lower exposure in ocular tissues. Further, this study emphasized the consideration of differences that are compound and disease model specific when extrapolating the data to other species.

4 Summary

Due to various complexities involved in ocular drug delivery that pertains to the uniqueness of ocular barriers, target site for therapy (anterior vs. posterior segment), and different routes of administration, ocular pharmacokinetics is distinct and more intricate than systemic pharmacokinetics. A good understanding of the properties of ocular tissues primarily that act as barriers and targets for drug delivery is essential to understand its interaction with the drug. As most of the ocular diseases afflict anterior or posterior tissues and given the distinct properties of various ocular tissues, ocular pharmacokinetics should be deliberated discretely for these two regions (anterior and posterior segments) in alignment with drug delivery.

Considerable advances have been made to understand the mechanism of drug delivery to anterior segment tissues following various administration routes. Several experiments conducted in various animals and using isolated tissues over these years have advanced the understanding of the barrier properties, identifying the targets, and optimizing the drug and delivery platform to achieve target pharmacokinetic profile. With regard to the anterior segment, investigation on the barrier property of the anterior tissues (cornea, conjunctiva, and anterior sclera) and the establishment of desired molecular properties to circumvent the barriers has resulted in designing smart delivery vehicles and in experimenting novel administration routes. In case of posterior segment, advances in the field of computational

sciences and statistical research have resulted in the development of in silico models that predict the half-lives of drugs based on the physicochemical properties. Innovation in the materials science has contributed to the birth of biodegradable implants that prolong the drug release for several months thereby drastically reducing the dosing frequency and improving patient compliance.

Regardless of these advancements, there are still unmet needs to further advance the ocular pharmacokinetics to the next level. For instance, the lack of allometric models to extrapolate the findings from preclinical species to human still exists despite the large number of studies carried out in various species. With the recent advancements in the novel intraocular delivery systems (including intracameral, subconjunctival, and intravitreal), another area that needs pharmacokinetic intervention is the development of IVIVC. Since there will be increasing demand for screening various prototype delivery systems during the development stage in order to identify the ideal delivery system with desired pharmacokinetic profile, an IVIVC model will be of esteem value in minimizing the number of preclinical/clinical studies required. Moreover, one of the main purposes of collecting pharmacokinetic information is to correlate with efficacy or safety data so that an optimal dose and dosing regimen can be established. In the absence of any such correlation, a standalone pharmacokinetic data can serve little purpose as linking with in vitro potency parameters involve assumptions that may not hold true in an in vivo setting. Thus, linking the pharmacokinetics to endpoints of interest (efficacy, safety, or biomarkers) through PKPD models is of great value in establishing the importance of drug exposure and its relevance to successful therapy.

References

Acheampong AA, Shackleton M, Tang-Liu DD, Ding S, Stern ME, Decker R (1999) Distribution of cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs. Curr Eye Res 18(2):91–103

Acheampong AA, Shackleton M, John B, Burke J, Wheeler L, Tang-Liu D (2002) Distribution of brimonidine into anterior and posterior tissues of monkey, rabbit, and rat eyes. Drug Metab Dispos 30(4):421–429

Ahmed I, Patton TF (1985) Importance of the noncorneal absorption route in topical ophthalmic drug delivery. Invest Ophthalmol Vis Sci 26(4):584–587

Akpek EK, Vittitow J, Verhoeven RS, Brubaker K, Amar T, Powell KD, Boyer JL, Crean C (2009) Ocular surface distribution and pharmacokinetics of a novel ophthalmic 1% azithromycin formulation. J Ocul Pharmacol Ther 25(5):433–439. doi:10.1089/jop.2009.0026

Asena L, Akova YA, Goktas MT, Bozkurt A, Yasar U, Karabay G, Demiralay E (2013) Ocular pharmacokinetics, safety and efficacy of intracameral moxifloxacin 0.5% solution in a rabbit model. Curr Eye Res 38(4):472–479. doi:10.3109/02713683.2012.763101

Attar M, Shen J, Ling KH, Tang-Liu D (2005) Ophthalmic drug delivery considerations at the cellular level: drug-metabolising enzymes and transporters. Expert Opin Drug Deliv 2 (5):891–908. doi:10.1517/17425247.2.5.891

Baranano DE, Kim SJ, Edelhauser HF, Durairaj C, Kompella UB, Handa JT (2009) Efficacy and pharmacokinetics of intravitreal non-steroidal anti-inflammatory drugs for intraocular inflammation. Br J Ophthalmol 93(10):1387–1390. doi:10.1136/bjo.2009.157297

Barza M, Lynch E, Baum JL (1993) Pharmacokinetics of newer cephalosporins after subconjunctival and intravitreal injection in rabbits. Arch Ophthalmol 111(1):121–125

Baum J, Barza M (1983) Topical vs subconjunctival treatment of bacterial corneal ulcers. Ophthalmology 90(2):162–168

Buitrago E, Hocht C, Chantada G, Fandino A, Navo E, Abramson DH, Schaiquevich P, Bramuglia GF (2010) Pharmacokinetic analysis of topotecan after intra-vitreal injection. Implications for retinoblastoma treatment. Exp Eye Res 91(1):9–14. doi:10.1016/j.exer.2010.03.009

Cheruvu NP, Amrite AC, Kompella UB (2009) Effect of diabetes on transscleral delivery of celecoxib. Pharm Res 26(2):404–414. doi:10.1007/s11095-008-9757-2

Chien DS, Homsy JJ, Gluchowski C, Tang-Liu DD (1990) Corneal and conjunctival/scleral penetration of p-aminoclonidine, AGN 190342, and clonidine in rabbit eyes. Curr Eye Res 9 (11):1051–1059. doi:10.3109/02713689008997579

Coco RM, Lopez MI, Pastor JC, Nozal MJ (1998) Pharmacokinetics of intravitreal vancomycin in normal and infected rabbit eyes. J Ocul Pharmacol Ther 14(6):555–563. doi:10.1089/jop.1998.14.555

Conrad JM, Robinson JR (1977) Aqueous chamber drug distribution volume measurement in rabbits. J Pharm Sci 66(2):219–224

Dey S, Patel J, Anand BS, Jain-Vakkalagadda B, Kaliki P, Pal D, Ganapathy V, Mitra AK (2003) Molecular evidence and functional expression of P-glycoprotein (MDR1) in human and rabbit cornea and corneal epithelial cell lines. Invest Ophthalmol Vis Sci 44(7):2909–2918

Dias CS, Mitra AK (2000) Vitreal elimination kinetics of large molecular weight FITC-labeled dextrans in albino rabbits using a novel microsampling technique. J Pharm Sci 89(5):572–578. doi:10.1002/(SICI)1520-6017(200005)89:5<572::AID-JPS2>3.0.CO;2-P

Durairaj C, Shah JC, Senapati S, Kompella UB (2009) Prediction of vitreal half-life based on drug physicochemical properties: quantitative structure-pharmacokinetic relationships (QSPKR). Pharm Res 26(5):1236–1260. doi:10.1007/s11095-008-9728-7

Durairaj C, Kadam RS, Chandler JW, Hutcherson SL, Kompella UB (2010) Nanosized dendritic polyguanidilyated translocators for enhanced solubility, permeability, and delivery of gatifloxacin. Invest Ophthalmol Vis Sci 51(11):5804–5816. doi:10.1167/iovs.10-5388

Durairaj C, Chastain JE, Kompella UB (2012) Intraocular distribution of melanin in human, monkey, rabbit, minipig and dog eyes. Exp Eye Res 98:23–27. doi:10.1016/j.exer.2012.03.004

Durairaj C, Shen J, Cherukury M (2014) Mechanism - based translational pharmacokinetic - pharmacodynamic model to predict intraocular pressure lowering effect of drugs in patients with glaucoma or ocular hypertension. Pharm Res 31(8):2095–2106. doi:10.1007/s11095-014-1311-9

Elena PP, Jauch A (1997) Ocular distribution of lomefloxacin 0.3% after a single instillation in the infected eye of pigmented rabbits. J Ocul Pharmacol Ther 13(6):551–558. doi:10.1089/jop.1997.13.551

Ficker L, Meredith TA, Gardner S, Wilson LA (1990) Cefazolin levels after intravitreal injection. Effects of inflammation and surgery. Invest Ophthalmol Vis Sci 31(3):502–505

Gaudreault J, Fei D, Rusit J, Suboc P, Shiu V (2005) Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration. Invest Ophthalmol Vis Sci 46 (2):726–733. doi:10.1167/iovs.04-0601

Ghate D, Edelhauser HF (2006) Ocular drug delivery. Expert Opin Drug Deliv 3(2):275–287. doi:10.1517/17425247.3.2.275

Grass GM, Robinson JR (1988) Mechanisms of corneal drug penetration. II: ultrastructural analysis of potential pathways for drug movement. J Pharm Sci 77(1):15–23

Hamalainen KM, Kananen K, Auriola S, Kontturi K, Urtti A (1997) Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera. Invest Ophthalmol Vis Sci 38(3):627–634

Hughes PM (2014) Intraocular pressure reduction with intracameral bimatoprost implants. Google Patents

Iyer MN, He F, Wensel TG, Mieler WF, Benz MS, Holz ER (2005) Intravitreal clearance of moxifloxacin. Trans Am Ophthalmol Soc 103:76–81, discussion 81–73

Iyer MN, He F, Wensel TG, Mieler WF, Benz MS, Holz ER (2006) Clearance of intravitreal moxifloxacin. Invest Ophthalmol Vis Sci 47(1):317–319. doi:10.1167/iovs.05-1124

Jay WM, Shockley RK, Aziz AM, Aziz MZ, Rissing JP (1984) Ocular pharmacokinetics of ceftriaxone following subconjunctival injection in rabbits. Arch Ophthalmol 102(3):430–432

Kidron H, Del Amo EM, Vellonen KS, Urtti A (2012) Prediction of the vitreal half-life of small molecular drug-like compounds. Pharm Res 29(12):3302–3311. doi:10.1007/s11095-012-0822-5

Kim H, Csaky KG, Chan CC, Bungay PM, Lutz RJ, Dedrick RL, Yuan P, Rosenberg J, Grillo-Lopez AJ, Wilson WH, Robinson MR (2006) The pharmacokinetics of rituximab following an intravitreal injection. Exp Eye Res 82(5):760–766. doi:10.1016/j.exer.2005.09.018

Kim SH, Csaky KG, Wang NS, Lutz RJ (2008) Drug elimination kinetics following subconjunctival injection using dynamic contrast-enhanced magnetic resonance imaging. Pharm Res 25(3):512–520. doi:10.1007/s11095-007-9408-z

Lee VH, Chien DS, Sasaki H (1988) Ocular ketone reductase distribution and its role in the metabolism of ocularly applied levobunolol in the pigmented rabbit. J Pharmacol Exp Ther 246 (3):871–878

Liu W, Liu QF, Perkins R, Drusano G, Louie A, Madu A, Mian U, Mayers M, Miller MH (1998) Pharmacokinetics of sparfloxacin in the serum and vitreous humor of rabbits: physicochemical properties that regulate penetration of quinolone antimicrobials. Antimicrob Agents Chemother 42(6):1417–1423

Lopez-Cortes LF, Pastor-Ramos MT, Ruiz-Valderas R, Cordero E, Uceda-Montanes A, Claro-Cala CM, Lucero-Munoz MJ (2001) Intravitreal pharmacokinetics and retinal concentrations of ganciclovir and foscarnet after intravitreal administration in rabbits. Invest Ophthalmol Vis Sci 42(5):1024–1028

Maltese A, Bucolo C (2003) Pharmacokinetic profile of topical flunarizine in rabbit eye and plasma. J Ocul Pharmacol Ther 19(2):171–179. doi:10.1089/108076803321637708

Maurice D (2001) Review: practical issues in intravitreal drug delivery. J Ocul Pharmacol Ther 17 (4):393–401. doi:10.1089/108076801753162807

Maurice DM, Mishima S (1984) Ocular pharmacokinetics. In: Sears ML (ed) Pharmacology of the eye. Springer, Heidelberg, pp 19–116. doi:10.1007/978-3-642-69222-2_2

Mayers M, Rush D, Madu A, Motyl M, Miller MH (1991) Pharmacokinetics of amikacin and chloramphenicol in the aqueous humor of rabbits. Antimicrob Agents Chemother 35 (9):1791–1798

Mikkelson TJ, Chrai SS, Robinson JR (1973) Altered bioavailability of drugs in the eye due to drug-protein interaction. J Pharm Sci 62(10):1648–1653

Mindel JS, Yablonski ME, Tavitian HO, Podos SM, Orellana J (1981) Dipivefrin and echothiophate. Efficacy of combined use in human beings. Arch Ophthalmol 99(9):1583–1586

Navratil T, Garcia A, Verhoeven RS, Trevino L (2015) Advancing ENV515 (travoprost) intracameral implant into clinical development: nonclinical evaluation of ENV515 in support of first-time-in-human phase 2a clinical study. Paper presented at the ARVO Annual Meeting

Nofsinger JB, Forest SE, Eibest LM, Gold KA, Simon JD (2000) Probing the building blocks of eumelanins using scanning electron microscopy. Pigment Cell Res 13(3):179–184

Nomoto H, Shiraga F, Kuno N, Kimura E, Fujii S, Shinomiya K, Nugent AK, Hirooka K, Baba T (2009) Pharmacokinetics of bevacizumab after topical, subconjunctival, and intravitreal administration in rabbits. Invest Ophthalmol Vis Sci 50(10):4807–4813. doi:10.1167/iovs.08-3148

Prausnitz MR, Noonan JS (1998) Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. J Pharm Sci 87(12):1479–1488

Proksch JW, Granvil CP, Siou-Mermet R, Comstock TL, Paterno MR, Ward KW (2009) Ocular pharmacokinetics of besifloxacin following topical administration to rabbits, monkeys, and humans. J Ocul Pharmacol Ther 25(4):335–344. doi:10.1089/jop.2008.0116

Rao CS, Schoenwald RD, Barfknecht CF, Laban SL (1992) Biopharmaceutical evaluation of ibufenac, ibuprofen, and their hydroxyethoxy analogs in the rabbit eye. J Pharmacokinet Biopharm 20(4):357–388

Review and Evaluation of Pharmacology and Toxicology Data (1999) http://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/021114_S000_BETAXON%200.5%25_PHARMR_P1.PDF. Accessed 25 May 2016

Schmidt SY, Peisch RD (1986) Melanin concentration in normal human retinal pigment epithelium. Regional variation and age-related reduction. Invest Ophthalmol Vis Sci 27 (7):1063–1067

Schoenwald RD (2003) Ocular pharmacokinetics and pharmacodynamics. In: Mitra AK (ed) Ophthalmic drug delivery systems, 2nd edn. Marcel Dekker, Inc., New York, pp 135–179

Schoenwald RD, Huang HS (1983) Corneal penetration behavior of beta-blocking agents I: physiochemical factors. J Pharm Sci 72(11):1266–1272

Schopf L, Enlow E, Popov A, Bourassa J, Chen H (2014) Ocular pharmacokinetics of a novel loteprednol etabonate 0.4% ophthalmic formulation. Ophthalmol Ther 3(1-2):63–72. doi:10.1007/s40123-014-0021-z

Shafiee A, Bowman LM, Hou E, Hosseini K (2013) Ocular pharmacokinetics of bimatoprost formulated in DuraSite compared to bimatoprost 0.03% ophthalmic solution in pigmented rabbit eyes. Clin Ophthalmol 7:1549–1556. doi:10.2147/OPTH.S48766

Shah SS, Denham LV, Elison JR, Bhattacharjee PS, Clement C, Huq T, Hill JM (2010) Drug delivery to the posterior segment of the eye for pharmacologic therapy. Expert Rev Ophthalmol 5(1):75–93. doi:10.1586/eop.09.70

Shen YC, Wang MY, Wang CY, Tsai TC, Tsai HY, Lee YF, Wei LC (2007) Clearance of intravitreal voriconazole. Invest Ophthalmol Vis Sci 48(5):2238–2241. doi:10.1167/iovs.06-1362

Shen YC, Wang MY, Wang CY, Tsai TC, Tsai HY, Lee HN, Wei LC (2009) Pharmacokinetics of intracameral voriconazole injection. Antimicrob Agents Chemother 53(5):2156–2157. doi:10.1128/AAC.01125-08

Shen J, Durairaj C, Lin T, Liu Y, Burke J (2014) Ocular pharmacokinetics of intravitreally administered brimonidine and dexamethasone in animal models with and without blood-retinal barrier breakdown. Invest Ophthalmol Vis Sci 55(2):1056–1066. doi:10.1167/iovs.13-13650

Shorstein NH, Winthrop KL, Herrinton LJ (2013) Decreased postoperative endophthalmitis rate after institution of intracameral antibiotics in a Northern California eye department. J Cataract Refract Surg 39(1):8–14. doi:10.1016/j.jcrs.2012.07.031

Sjoquist B, Stjernschantz J (2002) Ocular and systemic pharmacokinetics of latanoprost in humans. Surv Ophthalmol 47(Suppl 1):S6–S12

Sjoquist B, Basu S, Byding P, Bergh K, Stjernschantz J (1998) The pharmacokinetics of a new antiglaucoma drug, latanoprost, in the rabbit. Drug Metab Dispos 26(8):745–754

Stratford RE Jr, Lee VH (1985) Ocular aminopeptidase activity and distribution in the albino rabbit. Curr Eye Res 4(9):995–999

Tang-Liu DD, Liu SS, Weinkam RJ (1984) Ocular and systemic bioavailability of ophthalmic flurbiprofen. J Pharmacokinet Biopharm 12(6):611–626

Tang-Liu DD, Liu S, Neff J, Sandri R (1987) Disposition of levobunolol after an ophthalmic dose to rabbits. J Pharm Sci 76(10):780–783

Wang M, Liu W, Lu Q, Zeng H, Liu S, Yue Y, Cheng H, Liu Y, Xue M (2012) Pharmacokinetic comparison of ketorolac after intracameral, intravitreal, and suprachoroidal administration in rabbits. Retina 32(10):2158–2164. doi:10.1097/IAE.0b013e3182576d1d

Weijtens O, Schoemaker RC, Lentjes EG, Romijn FP, Cohen AF, van Meurs JC (2000) Dexamethasone concentration in the subretinal fluid after a subconjunctival injection, a peribulbar injection, or an oral dose. Ophthalmology 107(10):1932–1938

Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J (2006) DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res 34(Database issue):D668–D672. doi:10.1093/nar/gkj067

Zhang J, Wang L, Gao C, Zhang L, Xia H (2008a) Ocular pharmacokinetics of topically-applied ketoconazole solution containing hydroxypropyl beta-cyclodextrin to rabbits. J Ocul Pharmacol Ther 24(5):501–506. doi:10.1089/jop.2008.0015

Zhang T, Xiang CD, Gale D, Carreiro S, Wu EY, Zhang EY (2008b) Drug transporter and cytochrome P450 mRNA expression in human ocular barriers: implications for ocular drug disposition. Drug Metab Dispos 36(7):1300–1307. doi:10.1124/dmd.108.021121

Zhang L, Li Y, Zhang C, Wang Y, Song C (2009) Pharmacokinetics and tolerance study of intravitreal injection of dexamethasone-loaded nanoparticles in rabbits. Int J Nanomedicine 4:175–183

Ocular Drug Delivery

Burcin Yavuz and Uday B. Kompella

Contents

Abstract

Although the eye is an accessible organ for direct drug application, ocular drug delivery remains a major challenge due to multiple barriers within the eye. Key barriers include static barriers imposed by the cornea, conjunctiva, and retinal pigment epithelium and dynamic barriers including tear turnover and blood and lymphatic clearance mechanisms. Systemic administration by oral and

B. Yavuz
University of Colorado, Skaggs School of Pharmacy and Pharmaceutical Sciences, 12850 East Montview Blvd., C238-V20, Aurora, CO 80045, USA

Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA

U.B. Kompella (✉)
University of Colorado, Skaggs School of Pharmacy and Pharmaceutical Sciences, 12850 East Montview Blvd., C238-V20, Aurora, CO 80045, USA
e-mail: Uday.Kompella@ucdenver.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_84

parenteral routes is limited by static blood–tissue barriers that include epithelial and endothelial layers, in addition to rapid vascular clearance mechanisms. Together, the static and dynamic barriers limit the rate and extent of drug delivery to the eye. Thus, there is an ongoing need to identify novel delivery systems and approaches to enhance and sustain ocular drug delivery. This chapter summarizes current and recent experimental approaches for drug delivery to the anterior and posterior segments of the eye.

Keywords
Anterior segment • Implants • Microparticles • Nanomedicines • Ocular barriers • Ocular drug delivery • Ocular transporters • Posterior segment

1 Introduction

Ocular drug delivery systems or dosage forms range from the most common eye drops and other conventional formulations that are dosed daily to more complex implant systems that can be dosed once every few years. Conventional dosage forms like solutions, suspensions, emulsions, and ointments are only able to treat a limited number of ocular diseases. Ocular inserts and/or implants; preformed gels; in situ gels; microparticles; liposomes; nanotechnology-derived drug delivery systems such as nanoparticles, nanoemulsions, and nanomicelles; and the physical approaches to enhance drug delivery like iontophoresis and microneedles are some of the widely investigated ophthalmic drug delivery systems and approaches to meet unmet medical needs while overcoming ocular drug delivery challenges.

Each ocular tissue layer might act like a barrier based on drug physicochemical properties, drug carrier properties, and clearance mechanisms of a given route of administration. Thus, a delivery system or approach should be optimized for a given target tissue. For drug delivery purposes, the eye can be divided into two major segments, the anterior segment from the front of the eye to the lens and the posterior segment including eye tissues beyond the lens. These two different ocular regions are unique and face different challenges in drug delivery and should be dealt separately. Current studies are promising in terms of overcoming the challenges in treating various anterior and posterior segment diseases.

This chapter will briefly discuss the general considerations for ocular drug delivery and focus on drug delivery to both the anterior and posterior segments of the eye. The challenges, different application routes, and recent efforts to overcome these challenges will be elaborated for both segments separately.

2 General Considerations for Ocular Drug Delivery

A multitude of ocular diseases affect millions of individuals all over the world, and most of them have a significant negative impact on vision, leading to a decrease in patients' quality of life. The major ocular diseases include age-related macular

degeneration (AMD), diabetic retinopathy (DR), cataract, uveitis, and glaucoma, which can lead to blindness unless treated (Pascolini and Mariotti 2012).

The human eye is a 7.5 g globular structure with a diameter of approximately 24 mm, comprised of various tissues each presenting different features and playing a necessary role in vision (Hosoya et al. 2011). Since the eye is an extension of the central nervous systems, it is well protected from toxic materials through multiple barriers that inherently restrict drug delivery. Drug delivery barriers are specific depending on the target tissue and administration route (Gaudana et al. 2009, 2010).

One of the main problems with ocular drug delivery via conventional dosage forms is eye irritation that results in patient discomfort as well as reduced ocular bioavailability due to reflex tear flow. Most of the ophthalmological drugs are weak bases, and to enhance their solubility, they are usually formulated in an acidic pH, which may result in poor ocular diffusion due to the ionized state of the drug molecule. Another drug-related concern for ocular drug delivery is frequent dosing to maintain therapeutic amounts in the target tissue due to short drug residence time in the precorneal area. Drug administered in a drop is rapidly drained into the nose via the nasolacrimal ducts, resulting in unwanted systemic absorption and side effects.

Several factors need to be considered when designing an ocular drug delivery system that overcomes current limitations. These include improved dose accuracy, enhanced ocular bioavailability by overcoming static and dynamic barriers, and sustained and targeted drug delivery in order to enhance treatment efficacy and patient convenience (Macha and Mitra 2003).

2.1 Drug Administration Routes

Ocular barriers are generally specific for application route. The main administration routes for ocular drug delivery include topical, periocular, intraocular, and systemic.

Topical administration is the most common route for treating diseases of the anterior segment of the eye, due to ease of application, drug localization and adequate efficacy, and low cost. However, only about 30–50 μl of ophthalmic solution is delivered using a dropper, due to limited holding capacity of the precorneal area. The eye drop mixes with tears and drains rapidly from the eye surface via nasolacrimal drainage till it reaches approximately 7 μl, the normal resident tear volume in the eye. As a result of this and continuous tear replacement, drug on the eye surface is rapidly lost, and the remaining drug encounters permeability barriers, resulting in less than 5% dose delivery to the anterior segment of the eye for most therapeutic agents. Drug entering the intraocular tissues is rapidly cleared through turnover of aqueous humor and blood circulation. Therefore, frequent drug application is required to maintain adequate drug concentrations in the eye. In addition to low corneal permeability, short precorneal residence time is a critical rate-limiting factor for drugs to cross corneal barrier after topical instillation (Shell 1985; Lee and Robinson 1986; Hughes et al. 2005). Because of poor drug

delivery, topical administration is usually reserved for ocular surface and anterior segment diseases but not posterior segment diseases, although there are studies indicating that topical application can deliver drugs to the posterior segment of the eye (Furrer et al. 2009).

Oral and parenteral applications are the most common methods for systemic delivery, with the oral route being more convenient. Even though systemic administration might be useful in treating posterior segment eye diseases, high doses and frequent dosing may be required since there are various limitations including extensive drug dilution in the blood, low cardiac output to the eye, and blood–ocular barriers that restrict drug permeability. Furthermore, drugs administered by the systemic route are subjected to metabolism by the liver and clearance by the kidney, resulting in only a small quantity of the drug typically reaching the vitreous humor (Furrer et al. 2009; Duvvuri et al. 2003; Barar et al. 2008). High drug doses and frequent administrations usually result in systemic side effects.

Subconjunctival and sub-Tenon routes are commonly employed periocular routes for off-label drug dosing via injections. Other periocular modes of administration include posterior juxtascleral, peribulbar, and retrobulbar. The sclera has a large surface area (16.3 cm^2) (Olsen et al. 1998), and the subconjunctival space can expand and serve as a depot location for both anterior and posterior segment drug delivery (Ranta et al. 2010; Mac Gabhann et al. 2007).

When compared to noninvasive modes of administration including topical and oral routes, intraocular administration via injection or implantation is more difficult and uncomfortable for the patients; however, it is the only option to treat the diseases of the posterior segment of the eye in most cases. Intravitreal injection, one type of intraocular injection, gained widespread acceptance in the recent years with the commercial success of a few approved drug products. Other intraocular routes of administration include subretinal and suprachoroidal routes. Thus, intravitreal, periocular, subretinal, and suprachoroidal routes are the key routes that have been studied to overcome the drug delivery challenges where topical and systemic applications were not adequate (Gaudana et al. 2009; Raghava et al. 2004). Key advantages and disadvantages of the intraocular as well as conventional drug administration routes are summarized in Table 1.

2.2 Pharmacokinetic Considerations

Ocular pharmacokinetics including absorption, distribution, metabolism, and excretion are more complicated and harder to describe than systemic pharmacokinetics. This is due in part to the unique structure of the eye, various application routes, and formulation types that are used for ocular drug delivery. Since it is difficult to collect ocular pharmacokinetic data and data modeling may be difficult, the literature is very limited about ocular pharmacokinetics and mostly covers the measurement of drug levels in aqueous humor following ocular administration (Macha and Mitra 2003; Schoenwald 1990; Mishima 1981; Davies 2000). It is difficult to develop quantitative predictions for interspecies dose adjustments since

Table 1 Advantages and disadvantages of key routes for ocular drug delivery (modified from Gaudana et al. 2010)

Route	Advantages	Disadvantages
Topical	– Noninvasive – Self-administration possible – Patient convenience – Sustained delivery for a day is possible; inserts allow more prolonged release	– Low ocular bioavailability – Nasolacrimal drainage – Epithelial barriers – Not yet approved/effective for posterior segment
Systemic	– Noninvasive – Self-administration possible – Patient convenience	– Low ocular bioavailability – Blood–aqueous barrier – Blood–retinal barriers – Systemic toxicity and side effects
Periocular/ suprachoroidal	– Delivery possible for both anterior and posterior segments – Possible depot site	– Invasive – Patient inconvenience – Clearance by circulation – Retinal pigment epithelial (RPE) barrier for retinal delivery – Potential hemorrhage
Subretinal	– Useful for retinal gene delivery – Bypasses RPE barrier – Sustained retinal gene delivery	– Invasive – Patient inconvenience – Retinal detachment and risk of retinal damage
Intravitreal	– Effective retinal delivery – Sustained delivery up to about 3 years – Bypasses multiple ocular barriers	– 100% vitreal bioavailability – Invasive – Patient inconvenience

most of these studies were performed in rabbit eyes with different anatomical and physiological features (such as blinking rate, tear volume, and corneal dimensions) relative to human eyes and because there is little or no data available in human eyes. However, predictive models are being developed to estimate vitreal half-life of a new chemical entity (Durairaj et al. 2009a).

Drug physicochemical properties such as molecular weight, solubility, lipophilicity, and degree of ionization play an important role in drug absorption into the eye. The cornea is the most important barrier with a multilayered structure for drug absorption into the anterior segment; however, the conjunctiva is generally more permeable than the cornea. Elimination processes from the eye vary for different drugs. Tear drainage, aqueous humor turnover, and entry into systemic circulation from the eye tissues are some of the major elimination routes. These aspects and ocular pharmacokinetics are discussed in detail in another chapter.

2.3 Transporters in the Eye

Several transporters including influx and efflux transporters are present in the cornea, conjunctiva, retina and blood–ocular barriers, which may influence drug bioavailability (Gaudana et al. 2009; Macha and Mitra 2003). Modifications targeting these transporters might be an alternative approach to improve ocular bioavailability of drugs.

Efflux transporters tend to reduce cellular bioavailability by transporting drugs out of the cell. Key efflux transporters associated with ocular tissues are P-glycoprotein (P-gp) and multidrug resistance protein (MRP), which belong to the ATP-binding cassette (ABC) superfamily (Eytan and Kuchel 1999; Dey et al. 2003; Mannermaa et al. 2006). P-gp effluxes lipophilic drugs and prevents drug accumulation in the cells. P-gp is present in the cornea, conjunctiva, ciliary nonpigmented epithelium, iris, and retina (Saha et al. 1998; Wu et al. 1996). MRP works in a similar way as P-gp to efflux organic anions and their conjugates (Aukunuru et al. 2001; Steuer et al. 2005).

On the other hand, influx transporters that belong to the solute carrier (SLC) superfamily transport essential nutrients and xenobiotics across biologic membranes (Hosoya et al. 2005; Hosoya and Tachikawa 2012). Influx transporters include amino acid, peptide, vitamin, glucose, lactate, and nucleoside carriers. Designing prodrugs targeting these influx transporters has been an important approach for ocular drug delivery, and two key influx transporters are the amino acid and peptide transporters. Some amino acid transporters identified in ocular tissues include ASCT1, ASCT2, B(0,+), LAT1, and LAT 2 (Hosoya et al. 1997, 2005; Hosoya and Tachikawa 2012; Hosoya and Lee 1997; Jain-Vakkalagadda et al. 2003, 2004). Peptide transporters in the eye have also been investigated, and it was reported that PEPT1 and PEPT2 were detected in the corneal epithelium (Zhang et al. 2008; Xiang et al. 2009). In addition to these transporters, organic cation/anion, monocarboxylate, nucleoside, and vitamin transporters have been identified in various ocular tissues (Talluri et al. 2006; Janoria et al. 2006).

Both anterior and posterior segment tissues of the eye express various transporters, which are promising for the design of prodrugs for transporter-targeted drug delivery.

3 Drug Delivery to the Anterior Segment of the Eye

3.1 Recent Anterior Segment Drug Delivery Approaches

Various approaches have been studied in order to improve drug delivery across ocular tissues and enhance therapeutic efficacy of drugs intended for the treatment of anterior segment diseases such as dry eye syndrome, conjunctivitis, glaucoma, postoperative inflammation, and uveitis. Conventional dosage forms including solutions, suspensions, emulsions, and ointments are routinely used for treating anterior segment diseases. Viscous gels, mucoadhesive agents, prodrugs, and

nanosystems are some of the novel approaches employed to increase or sustain drug delivery and efficacy, to reduce systemic side effects, and to improve patient comfort and compliance. Some key novel dosage forms are further discussed below.

3.1.1 Mucoadhesive Formulations

Increasing the retention time in the precorneal area is one of the main approaches to enhance ocular bioavailability. Mucoadhesion, which refers to attachment to mucus either by hydrogen bonding or electrostatic binding with mucin layer, may influence drug absorption (Sigurdsson et al. 2013). The most common mucoadhesives employed in ocular formulations are water-soluble polymers that cannot cross ocular barriers such as the polyacrylic derivatives including carbomers and thiomers, xanthan gum, carrageenan, chitosan, and hyaluronic acid. Bioadhesive microspheres are also prepared to adhere to ocular mucin layer and prolong corneal contact time of any associated therapeutic agents (Gu et al. 1988; Ruponen and Urtti 2015; Kaur and Smitha 2002). Horvat et al. (2015) tested new hyaluronic acid (HA) derivatives for their mucoadhesive properties in ocular formulations (Horvat et al. 2015). Cross-linked sodium-, linear sodium-, and zinc-hyaluronate formulations of a nanosize were all characterized as potential ocular drug delivery systems. Another approach is to combine nanotechnology with mucoadhesive systems. As a recent example to this approach, Chaiyasan and co-workers have developed and characterized mucoadhesive chitosan–dextran sulfate nanoparticles for sustained ocular drug delivery. Based on the mucoadhesion and in vitro release studies, the system was reported as promising (Chaiyasan et al. 2013).

3.1.2 Gels: In Situ Gels

The main focus of the research is on in situ gels, which are solutions that start gelation upon contact with the ocular tissues via pH- or temperature-dependent activation. These systems have the advantage to be easily instilled as a regular eye drop and can provide prolonged retention time and sustained drug release with unique gelation properties. Gels and in situ gel-forming systems have been investigated to increase the retention time. Pilocarpine formulated in a gel has proved to be more effective than the solution form (Ticho et al. 1979). Sachinkumar et al. (2015) prepared a pH-triggered in situ gel formulation of norfloxacin using hydroxypropyl methylcellulose (HPMC) for the treatment of ocular infections. The system was tested in vitro and still requires in vivo studies to confirm in situ gelation properties (Sachinkumar et al. 2015).

Hydrogels have a variety of applications in ophthalmology including in situ gelling formulations, soft contact lenses, foldable intraocular lenses, and ocular adhesives for wound repair. High water content of hydrogels may be advantageous in preserving peptide/protein stability. Chemically cross-linked temperature-sensitive hydrogels that have high water content and retain transparency have been used as in situ forming gels (Kirchhof et al. 2015).

A dendrimeric hydrogel system has been developed using polyamidoamine (PAMAM) dendrimer G3.0 for delivery of antiglaucoma drugs brimonidine and

timolol maleate. Dendrimeric hydrogel was found to be mucoadhesive to mucin particles on the cornea. PAMAM dendrimers were linked with polyethylene glycol (PEG) acrylate to achieve in situ gelation upon UV light activation (Holden et al. 2012).

Yu et al. have developed a crossed-linked PEG in situ hydrogel for sustained bevacizumab delivery (Yu et al. 2014). The same group has also prepared cross-linked polysaccharide hydrogels using glycol chitosan and oxidized alginate for sustained bevacizumab release (Xu et al. 2013). Following in vitro and cell culture studies, both systems have been suggested to be promising for the treatment of intraocular neovascularization. Examples of in situ forming gels in the market include timolol maleate-loaded gellan gum-based product (Timoptic XE®) and another timolol maleate-containing formulation based on methyl cellulose, sodium citrate, and polyethylene glycol (Rysmon® TG) (Agrawal et al. 2012).

3.1.3 Prodrugs

Prodrugs are intended to be pharmacologically inactive or less-active derivatives of drug molecules, and ophthalmic prodrugs are typically designed to achieve improved drug delivery and/or therapeutic index. Following tissue entry, the prodrug is expected to be metabolized and produce the active form of the drug. Prodrugs are chemically synthesized to usually contain ester, amide, or other enzymatically cleavable chemical bonds. The enzymes in the ocular tissues play an important role in the conversion of prodrug to drug. Esterases and amidases are the most common enzymes in ocular tissues with high enzyme activity detected in the iris–ciliary body, cornea, and aqueous humor (Lee 1983; Stratford and Lee 1985). Latanoprost is a successful ocular prodrug with high ocular penetration that hydrolyzes via an esterase enzyme to produce the active form of the drug (Sjoquist and Stjernschantz 2002).

Several prodrug strategies including transporter-targeted and lipophilic ester prodrugs have been assessed to improve corneal and conjunctival permeability of drugs targeting the anterior segment. Some prodrug strategies for anterior segment drug delivery are summarized in Table 2.

3.1.4 Colloidal Drug Delivery Systems

Various types of colloidal dosage forms have been designed to increase precorneal residence time and anterior segment drug delivery. Some of these particles can interact with ocular mucosa and enhance permeability across the cornea and conjunctiva. Polymeric nanoparticles, nanomicelles, nanosuspensions, nanoemulsions, nanocrystals, liposomes, niosomes, cubosomes, and dendrimers are among the most studied drug delivery systems for anterior segment diseases such as dry eye, inflammation, glaucoma, uveitis, and retinopathies. Colloidal drug delivery systems offer the advantage of being able to deliver a variety of drugs (including macromolecules), providing stability for labile drugs and improving ocular bioavailability (Reimondez-Troitino et al. 2015a).

A number of studies focused on these systems in the last decade. These colloidal drug delivery systems have been classified and summarized in Table 3. Despite

Table 2 Summary of prodrug studies for anterior segment drug delivery

Drugs	Prodrugs	Outcome
Timolol (Chang et al. 1987)	Lipophilic esters	Increase in corneal permeation Decrease in systemic drug exposure
PGF2α (Chien et al. 1997)	Lipophilic esters	Increase in corneal permeation
Acyclovir (Hughes and Mitra 1993; Katragadda et al. 2006; Vadlapudi et al. 2012a, b, 2014)	Aliphatic acyl esters Amino acid prodrugs Peptide prodrugs Lipid prodrugs	Increase in corneal permeation Enhancement of stability Increase in efficacy Increase in cell accumulation
Ganciclovir (Tirucherai et al. 2002; Majumdar et al. 2005)	Lipophilic esters	Increase in solubility Increase in corneal permeation Increase in activity
Dexamethasone (Civiale et al. 2004)	Esters	Increase in corneal permeation
Cyclosporine A (Lallemand et al. 2005, 2007; Rodriguez-Aller et al. 2012)	Phosphate ester	Therapeutic concentrations in precorneal area immediately after application Increase in water solubility
Flurbiprofen (Shen et al. 2011)	Flurbiprofen axetil	Less irritation Increase in efficacy
Cannabinoid receptor ($CB_{1/2}$) agonist (Mainolfi et al. 2013)	Esters	Improved solubility Increased corneal permeation Enhanced ocular bioavailability
Resolvin E1 (de Paiva et al. 2012)	Methyl ester	Reduced corneal disruption

many promising results, reaching posterior segment via topically administered systems is still a challenge. There is still more to be investigated, especially regarding delivery of complex biomacromolecules to the eye.

3.1.5 Ocular Inserts and Implants for Anterior Segment Diseases

Ocular inserts and implants are designed to enhance the bioavailability and achieve sustained drug delivery. These systems can be placed under the eyelid, in the conjunctival cul-de-sac, anterior chamber, subconjunctival space, or episcleral region to deliver drugs to the anterior segment of the eye. They can be either in the biodegradable or nonbiodegradable form. Ocusert® was the first marketed ocular insert, which provides an extended therapeutic effect for a week with a low amount of pilocarpine. It consists of two ethylene–vinyl acetate copolymer membranes that control drug release to achieve zero-order kinetics (Ghate and Edelhauser 2006).

Anterior chamber implants can be placed in the aqueous humor. Subconjunctival/episcleral implants require a small incision in the conjunctiva. Surodex™ is a biodegradable anterior chamber insert made of poly(lactic-co-glycolic acid) (PLGA) and provides sustained-release dexamethasone for about

Table 3 Colloidal systems for anterior segment drug delivery

Delivery system	Drug	Formulation type/component	Animal models	Results	References
Nanoparticles	Ibuprofen	Eudragit RS100®	Rabbits with ocular trauma	Increase in AH concentration Good in vivo tolerance	Pignatello et al. (2002)
	Flurbiprofen	PLGA, PCL		Increase in ocular bioavailability	Valls et al. (2008)
		PLGA, poloxamer 188	Rabbits with inflammation	Increase in activity	Vega et al. (2006)
		Chitosan	Rabbits	Increase in corneal AUC Increase in retention time	Luo et al. (2011)
	Indomethacin	Chitosan	Rabbits with corneal ulcer	Increase in AH and corneal concentrations Corneal healing Good in vivo tolerance	Badawi et al. (2008)
		PCL	Rabbits	Increase in corneal, AH, and CB concentrations	Calvo et al. (1996)
	Cyclosporine A	Chitosan, cholesterol-conjugated chitosan	Rabbits	Increase in retention time Increase in corneal and conjunctival concentrations	De Campos et al. (2001), Hu et al. (2006)
		Hyaluronic acid, PCL	Rabbits	Increase in corneal, AH, and CB concentrations	Yenice et al. (2008)
		Cysteine–PEG monostearate	Rabbits	Increase in concentration and duration of exposure for the cornea, AH, CB, and conjunctiva Good in vivo tolerance	Shen et al. (2010)
		Propylene glycol	Rabbits	Eye tissue concentrations similar to Restasis® Good in vivo tolerance	Khan et al. (2012)

		Carbopol®–PLGA, Eudragit®–PLGA	Rabbits	Increase in tear AUC	Aksungur et al. (2011)
	Rapamycin	Chitosan, PLA	Rabbits with inflammation	Decrease in inflammation	Yuan et al. (2008)
	Prednisolone, gatifloxacin	Eudragit RS100® Hyaluronic acid	Rabbits	Increase in ocular bioavailability	Ibrahim et al. (2010)
	Levofloxacin	PLGA	Rabbits	Increase in corneal retention time Good in vivo tolerance	Gupta et al. (2011)
	Amikacin	PACA, dextran	Rabbits	Increase in corneal and AH concentrations	Losa et al. (1991)
	MUC5AC Plasmid DNA	Cationized gelatin	Rabbits; mice with dry eye	Conjunctival transfection of MUC5AC Increase in tear production	Konat Zorzi et al. (2011), Contreras-Ruiz et al. (2013)
	Pilocarpine	PLGA	Rabbits	Increase in miotic response and duration	Nair et al. (2012)
	Metipranolol	PCL	Rabbits	Decrease in systemic absorption	Losa et al. (1993)
	Dorzolamide HCl	Chitosan	Rabbits	Decrease in systemic absorption Increase in precorneal residence	Katiyar et al. (2014)
	Timolol maleate Dorzolamide HCl	Hyaluronic acid, chitosan	Rabbits with elevated intraocular pressure	Increase in activity Decrease in systemic absorption Good in vivo tolerance	Wadhwa et al. (2010)

(continued)

Table 3 (continued)

Delivery system	Drug	Formulation type/component	Animal models	Results	References
	Brimonidine tartrate	Eudragit®	Rabbits with elevated intraocular pressure	Increase in activity and duration of residence/activity	Bhagav et al. (2011)
Nanomicelles	Dexamethasone	NIPAAM	Rabbits with inflammation	Increase in activity and duration of activity	Rafie et al. (2010)
		Polyhydroxyethylaspartamide	Rabbits	Increase in AH AUC	Civiale et al. (2009)
		Chitosan	Rabbits	Increase in anterior eye tissue AUC	Pepic et al. (2010)
		Pluronic/chitosan	Rabbits	Increase in bioavailability	Pepic et al. (2010)
	Ketorolac	NIPAAM	Rabbits with inflammation	Increase in efficacy	Gupta et al. (2000)
	Pilocarpine	Pluronic F127	Rabbits	Increase in AUC and duration of activity	Pepic et al. (2004)
	Voclosporin Dexamethasone Rapamycin	Vitamin E Octoxynol-40	Rabbits	Increase in bioavailability	Mitra et al. (2010)
	Cyclosporine A	Polyoxyl stearate 40	Rabbits	Increase in anterior tissue AUC	Kuwano et al. (2002)
		MPEG–hexPLA	Rabbits	Good in vivo tolerance	Di Tommaso et al. (2011)
	Plasmid DNA with lacZ gene	PEO–PPO–PEO	Mice and rabbits	Increase in β-gal activity Decrease in corneal apoptosis	Tong et al. (2007)
Nanoemulsions	Indomethacin	Negatively and positively charged	Rabbits	Increase in AH, scleral, and retinal concentrations Good in vivo tolerance	Klang et al. (2000)

	Diclofenac	N-Octenylsuccinate starch	Porcine cornea (ex vivo)	Increase in corneal permeability	Baydoun and Muller-Goymann (2003)
	Flurbiprofen axetil	Castor oil, Tween 80	Rabbits with uveitis	Increase in efficacy	Shen et al. (2011)
	Prodrug pilocarpine Dorzolamide HCl	Soybean oil, lecithin	Rabbits	Increase in activity and duration of activity	Sznitowska et al. (1999)
	Tetrahydrocannabinol	–	Rabbits (normal and with elevated intraocular pressure)	Increase in activity Good in vivo tolerance	Muchtar et al. (1992)
	Cyclosporine A	Kelcogel®	Rabbits	Increase in ocular bioavailability	Gan et al. (2009)
Nanosuspensions	Indomethacin	Sesame oil	Human subjects	Increase in AH concentration	Sanders et al. (1983)
	Rofecoxib	Polystyrene, Poloxamer 407 Hydroxypropyl methyl cellulose	Rabbits	Increase in anterior chamber concentration	Santipharp and Laman (2008)
	Diclofenac Cyclosporine A	Sophisen	Human subjects	Increase in precorneal residence time Increase in tear production	Quintana-Hau et al. (2005)
	Prednisolone Hydrocortisone Dexamethasone	Pluronic F68	Rabbits	Increase in AH concentration	Kassem et al. (2007)
Liposomes	Acyclovir	–	Rabbits	Increase in corneal penetration	Law et al. (2000)
	Ganciclovir	–	Rabbits	Increase in corneal penetration	Shen and Tu (2007)
	Dexamethasone	Human serum albumin	Mice with uveoretinitis	Increase in ocular concentration	Arakawa et al. (2007)
	Fluconazole	–	Rabbits with keratomycosis	Increase in activity	Habib et al. (2010)

(continued)

Table 3 (continued)

Delivery system	Drug	Formulation type/component	Animal models	Results	References
	Ciprofloxacin hydrochloride	Chitosan coated	Rabbits with conjunctivitis	Increase in activity	Mehanna et al. (2010)
			Rabbits	Increase in ocular residence time Good in vivo tolerance	Abdelbary (2011)
	Diclofenac	Chitosan coated	Rabbits	Increase in AUC and prolonged retention Good in vivo tolerance	Li et al. (2009)
	Cyclosporine A	Supercritical fluid mediated	Rabbits with dry eye	Increase in tear AUC Increase in tear production	Karn et al. (2014)
	Tropicamide	–	Rabbits	Increase in residence time Increase in mydriatic activity	Nagarsenker et al. (1999)
	Pilocarpine HCl Timolol Brimonidine tartrate	–	Rabbits with elevated intraocular pressure	Sustained decrease in intraocular pressure	Monem et al. (2000)
	Ibuprofen	Silk fibroin-coated liposomes	Rabbit cornea (ex vivo)	Sustained drug release Increase in corneal transport No detectable toxicity	Dong et al. (2015)
Cubosomes	Dexamethasone	Poloxamer 407 Carbopol 974 Carboxy methyl cellulose sodium	Rabbits	Increase in corneal permeability Increase in AH AUC	Gan et al. (2010)
Niosomes	Brimonidine tartrate	–	Rabbits	Sustained decrease in intraocular pressure	Maiti et al. (2011)

	Brimonidine tartrate Timolol Acetazolamide	–	Rabbits with elevated intraocular pressure	Sustained decrease in intraocular pressure	Prabhu et al. (2010)
Dendrimers	Gatifloxacin	Polyguanidilyated dendrimers	Rabbits	Increase in corneal and conjunctival AUC	Durairaj et al. (2010)
	Carteolol	Phosphorous dendrimers	Rabbits	Increase in AH concentration Good in vivo tolerance	Spataro et al. (2010)
	Pilocarpine nitrate Tropicamide Brimonidine Timolol maleate	PAMAM dendrimers	Rabbits	Increase in bioavailability Sustained decrease in intraocular pressure	Vandamme and Brobeck (2005)

PCL poly(e-caprolactone), *PEG* polyethylene glycol, *PLGA* poly(lactic-co-glycolic acid), *PLA* poly(lactic acid), *PEO* poly(ethylene oxide), *PACA* poly(alkyl cyanoacrylate), *PPO* poly(propylene oxide), *NIPAAM* (N-isopropylacrylamide), *MPEG–hexPLA* methoxy poly(ethylene glycol)–hexylsubstituted poly (lactide), *PAMAM* polyamidoamine, *AH* aqueous humor, *CB* ciliary body

Based in part on Reimondez-Troitiño et al. (2015b), Thrimawithana et al. (2011), Gaudana et al. (2009), and the references listed in the table

10 days. It was developed for the treatment of postoperative inflammation following cataract surgeries (Kuno and Fujii 2011).

LX201 (Lux Biosciences) is a cyclosporine A-loaded silicone matrix episcleral implant designed to sustain drug release for a year in order to prevent corneal transplant rejection. A study aimed at a phase III trial (identification number NCT00447187) was terminated in 2012. Another cyclosporine A implant study for LX201 employed subconjunctival implantation that would not affect neovascularization following keratoplasty (Bock et al. 2014). It was also reported that cyclosporine A-loaded PLGA nanoparticles and poly[e-caprolactone] (PCL) subconjunctival implants were prepared for dry eye syndrome treatment, and they were able to extend the drug release up to 2 months and provided faster healing in dry eye-induced mice (Pehlivan et al. 2015). Pfizer Inc. collaborated on a PLGA subconjunctival insert for sustained-release latanoprost for glaucoma treatment; however, the phase I/IIa (NCT01180062) study was terminated due to inadequate supply of inserts.

Ang (2014) has developed prednisolone acetate-loaded PCL microfilms to be implanted subconjunctivally for uveitis treatment and has reported that the formulation was effective in a rabbit uveitis model (Ang 2014). Wong (1989) published a patent (US 7846468) for immunosuppressive biodegradable ocular implants using PLGA and HPMC against transplant rejection. It was reported that these systems were able to prevent allograft rejection in a rat model when implanted into the anterior chamber (Wong 1989).

Freeze-dried mini tablets as ocular inserts are another recent approach that presents several advantages such as easy/noninvasive application, increased corneal residence time, and reduced drug loss due to lacrimation. Cellulose derivatives, acrylates, and chitosan are the most commonly employed polymers for ocular mini tablet formulations (Moosa et al. 2014).

3.1.6 Punctal Plugs

Punctal plugs are small biocompatible implants used for dry eye treatment by insertion of the plug into tear ducts or puncta to block tear drainage. Punctal plugs may offer advantages such as being noninvasive and the ability to maintain sustained drug release. Silicone, hydroxyethyl methacrylate, and polycaprolactone were some of the materials used to prepare punctal plugs, but they require removal after drug release (Kompella et al. 2010). Drug release from punctal plugs is usually diffusion controlled, and the drug can be loaded in punctal plugs in various forms including solutions, suspensions, colloids, etc. One of the common designs for punctal plug drug delivery is loading drug to an impermeable core and releasing it from the cross section that is in contact with the eye surface and tears. An alternative approach is coating the plug with drug solution; however, drug loading might be low due to the small surface area (Yellepeddi et al. 2015).

Phase II studies were conducted for latanoprost and bimatoprost punctal plug formulations (QLT Inc. and Vistakon Pharmaceuticals) for glaucoma and ocular hypertension (Kuno and Fujii 2011). The system used for latanoprost is also being studied for the anti-allergy drug olopatadine. Another phase I study assessed

sustained-release moxifloxacin punctal plugs to prevent conjunctivitis after cataract surgery. This system achieved 7-day drug release as a potential alternative to topical antibiotic drops (Chee 2012). Gupta et al. (2011) reported a hydroxyl ethyl methacrylate punctal plug system loaded with cyclosporine A microparticles for dry eye treatment. The plug was covered with impermeable silicone shell and was able to release drug for over 3 months near zero-order kinetics (Gupta and Chauhan 2011).

Overall, drug-loaded punctal plugs are promising for sustained drug delivery to the eye surface. On the other hand, their use is associated with some complications such as conjunctivitis, corneal abrasion, distal lachrymal system blockage, excessive tear production (epiphora), and plug extrusion. These complications may be influenced by the design, size, and insertion method of the plug (Taban et al. 2006; Bourkiza and Lee 2012). It is believed that the experience gained from previous studies will lead to new plugs with less complications for anterior segment drug delivery.

3.1.7 Contact Lenses

Contact lenses provide an alternative approach for sustained drug delivery on the ocular surface and beyond. Polymethyl methacrylate was the first widely used polymer for the production of contact lenses, which were not able to allow adequate oxygen permeation for the cornea and had to be removed at night. This was a limitation for the use of contact lenses as a long-term drug delivery system. Highly oxygen-permeable silicone hydrogel contact lenses have overcome this issue, and contact lenses are now more promising as drug delivery systems (Sedlacek 1965; Chauhan 2015; Lu et al. 2013).

Pilocarpine-soaked contact lenses were the first example of these systems, and it was reported to provide reduction in intraocular pressure for a few hours with the equivalent efficacy of an eye drop (Hillman 1974). Cromolyn sodium, dexamethasone sodium phosphate, ketorolac tromethamine, ketotifen fumarate, and natamycin contact lenses were subsequently tested in vitro (Karlgard et al. 2003; Phan et al. 2014). Loading colloid nanoparticles and molecular imprinting have also been investigated as improved drug loading techniques to achieve prolonged release, since the commercial contact lenses are able to release for only 1–2 h (Jung and Chauhan 2012). It was shown that latanoprost- (Mohammadi et al. 2014) and norfloxacin-imprinted (Carmen et al. 2006) 2-hydroxyethylmethacrylate (HEMA) contact lenses were able to provide extended release as well as ciprofloxacin-imprinted silicone hydrogels (Hui et al. 2012).

Vitamin E-loaded contact lenses can provide extended drug release by forming additional diffusion barriers (Peng et al. 2012). Vitamin E loading is also effective for combination therapy. In a recent study, timolol and dorzolamide contact lenses with vitamin E coating were prepared to achieve simultaneous extended release of the two drugs, and the results indicated that the system was able to reduce intraocular pressure at a lower drug dose (Hsu et al. 2015). The main limitations with contact lens delivery systems are their higher cost relative to eye drops and ultimate acceptance by patients and clinicians.

3.1.8 Intraocular Lenses (IOL)

Drug-loaded intraocular lenses were developed as an alternative to the currently used postoperative drug products. Biodegradable polymer rings with triamcinolone acetonide were developed, attached to the disk of IOL, and implanted in New Zealand white rabbits, and the results indicated inflammation reduction in inflammatory signals in aqueous humor up to 7 weeks (Eperon et al. 2008). In another study, multilayer-coated intraocular lenses were designed for sustained drug delivery (Shukla et al. 2011).

A nonbiodegradable capsule drug ring has been investigated to serve as a refillable drug depot for multiple drugs. The system is to be placed around the intraocular lens after cataract surgery and can accomplish either anterior or posterior segment drug delivery depending on where the semipermeable membrane's location is in the capsule drug ring. This system has been studied using bevacizumab and showed nearly zero-order release kinetics (Molokhia et al. 2010).

3.1.9 Transcorneal Iontophoresis

Iontophoresis is a noninvasive technique that employs electric current in contact with eye tissues to deliver drug molecules across a biological membrane. An iontophoresis device consists of two electrodes: one donor that holds the drug solution and one receiver to close the electrical circuit and enhance drug delivery either by electrophoresis, electroosmosis, or electroporation. Transcorneal iontophoresis can deliver dugs to the anterior chamber, whereas transscleral iontophoresis may deliver drugs to the posterior segment. The efficiency of iontophoresis usually depends on the charge of the drug, electrode placement, and duration of pulse (Molokhia et al. 2008, 2013).

Eyegate® developed a transcorneal iontophoretic system made of soft silicone rubber and tungsten electrodes. The drug solution is placed in the tungsten electrode annularly well and flows through the silicone tubes. Dry eye, scleritis, and anterior uveitis drug indications have been assessed with this system in clinical trials (Halhal et al. 2004). Antibiotics (gentamicin, ciprofloxacin, and tobramycin) delivered via iontophoresis decreased the bacterial colony level when compared to corresponding eye drop applications (Cohen et al. 2012; Hobden et al. 1990). Dexamethasone when delivered using transcorneal iontophoresis exhibited greater corneal penetration than positively charged antibiotics. Using iontophoresis, riboflavin can be delivered across the intact corneal epithelium (epi-on technique) to induce collagen cross-linking in order to treat keratoconus (Bikbova and Bikbov 2014). This technique significantly reduces the application time for riboflavin, and it might be an alternative keratoconus treatment without removing the corneal epithelium. Delivery of macromolecules such as Galbumin, bevacizumab, and FITC dextrans can also be elevated, based on in vitro transcorneal iontophoresis (Molokhia et al. 2009; Chopra et al. 2010; Nicoli et al. 2009).

4 Drug Delivery to the Posterior Segment of the Eye

4.1 Barriers, Challenges, and Routes of Administration for Posterior Segment Drug Delivery

Posterior segment of the eye includes the sclera, choroid, retinal pigment epithelium, retina, optic nerve, and vitreous humor. Posterior segment diseases such as age-related macular degeneration (AMD), macular edema, diabetic retinopathy (DR), and posterior uveitis are eye diseases that lead to blindness. These diseases are becoming more common with the aging of the general population. Thus, there is a growing need to develop new therapies and delivery approaches to treat diseases of the posterior segment or back of the eye. Posterior segment drug delivery is more difficult than anterior segment delivery, due to the highly protected structure of the back of the eye with static (sclera, RPE, and blood capillary endothelial cell walls) and dynamic barriers (blood and lymph circulation). The delivery route depends on the drug molecule, dosage form, and the target tissue (Gaudana et al. 2009; Ghate and Edelhauser 2006).

Drug delivery to the retina and vitreous humor is limited and generally ineffective with an eye drop, due to the anatomical and physiological barriers. For retinal drug delivery following systemic administration, drugs must cross the blood–ocular barriers, which separate the eye from the rest of the body. Blood–ocular barriers consist of two key components: blood–aqueous barrier and blood–retinal barrier. Both these barriers are comprised of epithelial and endothelial tight junctions that limit drug transport (Macha and Mitra 2003), with the blood–retinal barrier being the limitation for back-of-the-eye drug delivery following systemic administration. Systemic administration requires large doses for therapeutic effects, due to drug dilution in blood prior to reaching the retina, low cardiac output to the eye, and the presence of strong blood–retinal barriers. Thus, the extent of dose delivery by conventional routes to the back of the eye is very limited.

Periocular dosing interfaces the drug with the sclera on one side and the conjunctiva on the other side (Raghava et al. 2004). While the episclera is vascularized, the sclera is the poorly vascularized white part of the globe that contains collagen fibers and mucopolysaccharides. Drug permeability across the sclera decreases with increasing molecular weight and lipophilicity; additionally, drug surface charge affects permeability since positive charges can interact with the negatively charged scleral matrix (Kim et al. 2007; Cruysberg et al. 2002; Dunlevy and Rada 2004). A perioculary dosed drug and particles can be cleared by vascular or lymphatic circulations (Amrite et al. 2008; Amrite and Kompella 2005; Cheruvu et al. 2008).

Suprachoroidal dosing interfaces the drug with the choroid on one side and the sclera on the other side. The choroid is a vascular tissue underlying Bruch's membrane that provides nutrients to the RPE and the retina. While the thickness

of Bruch's membrane increases with age, choroid thickness decreases with age (Spraul et al. 1999). The changes in thickness may affect drug permeability across these barriers. Furthermore, lipophilic drugs may bind to the pigment of choroid and may not reach inner ocular tissues such as the retina (Cheruvu et al. 2008; Cheruvu and Kompella 2006). Nano- and microparticles can potentially reside in the suprachoroidal space to allow prolonged drug delivery (Patel et al. 2012).

Subretinal route interfaces the drug with the retina on one side and the RPE on the other side, ideally suited to treat retinal degenerative diseases using gene therapies (Ghazi et al. 2016; Hauswirth et al. 2008). However, the safety of this route of administration needs further investigation.

Intravitreal injection allows placement of 100% of the dose in the back of the eye. Thus, intravitreal injections are the mainstay at the moment for treating back-of-the-eye diseases. However, the inner limiting membrane, which separates the retina and the vitreous humor, can be a barrier for drug diffusion, particularly macromolecules. As a result, retinal delivery of macromolecules with 76 kDa and larger molecular weight is limited (Jackson et al. 2003). Also, expression of efflux pumps such as P-gp and MRP in eye tissues may restrict retinal delivery of small molecules. Intravitreally dosed drugs are eliminated along the anterior pathway via the aqueous humor or restricted from reaching retinal cells by the inner limiting membrane, as mentioned above (Pederson 2006). Furthermore, drug elimination by the posterior segment tissues is another factor limiting retinal drug exposure. Due to the barriers present in the eye, the vitreal half-life of a molecule can be prolonged by increasing its molecular size (Durairaj et al. 2009a). Additionally, injection of a drug suspension and increasing the dose number of a suspension are suitable approaches to increase the persistence of drug molecules injected in the vitreous humor (Durairaj et al. 2009a, b). Drug and dosage form physicochemical properties, interaction with solute/efflux transporters, site of administration, and pathophysiology all influence drug delivery to the posterior segment.

4.2 Penetration Pathways for Posterior Segment Drug Delivery

Penetration pathways to the posterior segment of the eye, for an eye drop application, are summarized in Table 4. Non-corneal route is generally deemed the most efficient for back of the eye drug delivery among the pathways listed, although the bioavailability from a drop is not significant to be typically effective in the back of the eye. By injecting the drug at various depths (e.g., periocular, suprachoroidal, subretinal, and intravitreal with increasing depths of injection into the vitreous humor), as opposed to drops on a surface, some or all of the barriers for posterior segment delivery can be overcome. The preferred administration route is dependent on drug and dosage form, physicochemical properties, and disposition (Ahmed and Patton 1985).

The current first choice for posterior segment delivery is intravitreal administration, which bypasses the corneal, conjunctival, scleral, choroidal, RPE, blood–tissue, and lens barriers to reach the vitreous humor. However, the risks involved

Table 4 Key pathways of drug delivery to tissues of the posterior segment of the eye from an eye drop

Non-corneal pathway	Tears → conjunctiva → sclera → choroid → RPE → neural retina → vitreous humor
Systemic recirculation from one eye to the other eye	Tears → conjunctival/choroidal/retinal vessels → systemic circulation → contralateral ocular circulation → intraocular tissues → vitreous humor of the contralateral eye
Corneal pathway	Tears → cornea → aqueous humor → posterior chamber/ lens → vitreous humor → neural retina
Uveal pathway	Tears → cornea → aqueous humor → sclera/ choroid → RPE → neural retina → vitreous humor

Injecting the drug in a deeper layer can bypass the preceding barriers

with repeated injections and low patient compliance are leading to the development of slow-release systems as well as assessment of other routes including subretinal, suprachoroidal, and periocular applications including subconjunctival, sub-Tenon, peribulbar, and retrobulbar (Raghava et al. 2004; Eljarrat-Binstock et al. 2010).

4.3 Recent Posterior Segment Drug Delivery Approaches

Even though the anterior segment medications contribute the most to the number of currently marked ophthalmic drug products, the drug product market for the posterior segment is rapidly growing with the development of innovative new molecular therapies and delivery systems. The validated VEGF target alone for wet AMD resulted in three approved intravitreally injectable anti-VEGF products to date: Macugen®, Lucentis®, and Eylea®, with Avastin®, a fourth product being compounded and used off-label. As new therapeutic agents enter the back-of-the-eye market, there is continued effort in developing new drug delivery approaches in order to allow noninvasive dosing, to reduce dosing frequency with invasive approaches, and to improve drug efficacy and safety. Penetration enhancers, prodrugs, and iontophoresis are some of the strategies employed to increase drug flux and enhance bioavailability, whereas colloidal delivery systems, gels, inserts, implants, and intraocular refillable devices are being investigated to achieve sustained release and reduce application frequency. A few noteworthy slow-release systems approved for sustained delivery of small molecule drugs in the back of the eye include Vitrasert®, Retisert®, Ozurdex®, and Iluvien®, with drug release durations ranging from approximately 6 months to 3 years.

4.3.1 Colloidal Dosage Forms for Posterior Segment Drug Delivery

Colloidal dosage forms like nanoparticles, nanogels, liposomes, and dendrimers have been evaluated for drug delivery to the posterior segment as well as the anterior segment of the eye. Since drug loading in these systems is generally low, they might be limited to drugs that are effective at low therapeutic doses.

Polymeric nanoparticles have been studied extensively for ocular drug delivery, and the FDA-approved polymer PLGA is one of the most investigated materials in the eye, in addition to polyvinyl alcohol, chitosan, and albumin (Kompella et al. 2013).

Nanogels are hydrogels that swell in water to form compactly packed nanoparticles, and they can be used for controlled release of hydrophilic or lipophilic compounds. Nanogel drug release kinetics may be controlled by pH and temperature. Cationic nanogels have been investigated for gene delivery (Vinogradov et al. 2004).

Dendrimers or highly branched polymers are also among the nanotechnology-based delivery systems studied for posterior segment drug delivery. Dendrimers can be interfaced with drug molecules either covalently or non-covalently. Polyamidoamine (PAMAM) dendrimers are the most studied dendrimers for drug and gene delivery, and they are available in a number of generations or sizes and surface charges. Kang et al. (2009) complexed carboplatin with PAMAM dendrimers for periocular delivery and showed that these complexes can reduce retinoblastoma tumor growth (Kang et al. 2009). In a recent study, PAMAM dendrimers with anionic and cationic charges were investigated for retinal dexamethasone delivery via topical and subconjunctival applications. It was reported that these systems were able to improve corneal and scleral permeability and provide higher ocular bioavailability; however, drug loading in these systems was limited (Yavuz et al. 2015). PAMAM–triamcinolone acetonide conjugates with 21% drug loading were also prepared, and cell culture studies indicated increased anti-inflammatory activity of triamcinolone acetonide (Kambhampati et al. 2015).

Liposomes are vesicular systems with various sizes in the range of nanometers to micrometers, composed of one or more phospholipid bilayers segregated by aqueous layers. Liposomes offer many advantages for drug delivery since they can encapsulate both hydrophilic and hydrophobic dugs as well as ionic molecules by using cationic or anionic lipids. It was reported that intravitreally injected liposomal formulations caused minimal toxicity while providing prolonged vitreal residence time (Peyman et al. 1989).

In some studies, macromolecules were delivered to the back of the eye following topical dosing with liposomes. In one study, plasmid DNA-loaded liposomes were found to express genes in retinal ganglion cells following topical application in a rat model (Matsuo et al. 1996). Coating diclofenac-loaded liposomes with hydrophobic PVA enhanced diclofenac delivery to the retina–choroid after topical instillation in rabbits (Fujisawa et al. 2012). Annexin A5-functionalized liposomes enhanced delivery of bevacizumab to the vitreous humor and retina following topical instillation to rats and rabbits (Davis et al. 2014). While these studies are promising, more confirmatory studies and mechanistic studies are needed to establish the therapeutic potential of liposomes in achieving macromolecule efficacy in the back of the eye.

Micelles are formed at concentrations above the critical micellar concentration of a substance, and they typically consist of monolayers of amphiphilic molecules that form a core and a corona. Based on the properties of the vehicle or continuous

medium and the properties of the amphiphilic compound, standard, reverse, and unimolecular micelles can be formed. These micelles can be formed at a very small size, typically less than 100 nm. Polyethylene glycol coating of the corona reduces micelle aggregation, and they have the potential for posterior segment drug delivery (Trivedi and Kompella 2010; Trivedi et al. 2012).

4.3.2 Prodrugs for Posterior Segment Drug Delivery

Prodrug approach is potentially useful in enhancing drug delivery to the posterior segment as well as the anterior segment. Lipophilic esters with increased permeability are among the most widely assessed prodrugs for ophthalmic drug delivery. In addition to the topical route, prodrugs can be dosed by various routes including intravitreal and periocular routes. Prodrugs can be designed to preferentially traverse solute transporters in the tissue barriers of the eye. Furthermore, incorporation of prodrugs within polymeric carriers may provide controlled drug delivery to the retina and vitreous (Eljarrat-Binstock et al. 2010).

Transporter-targeted gatifloxacin prodrugs have been prepared for topical application, and organic cation transporter, monocarboxylate transporter, and ATB transporters were targeted for enhanced drug delivery to the back of the eye. Ex vivo transport studies were performed against the cornea and sclera–choroid–RPE, as well as in vivo studies in rats. It was reported that prodrug increased solubility and enhanced organic cation transporter-mediated delivery of gatifloxacin (Vooturi et al. 2012). Posterior segment distribution of nepafenac (which is a prodrug for amfenac) has been studied in rabbit and monkey models following topical administration. The study suggested that nepafenac and amfenac were able to distribute in posterior segment tissues via transconjunctival/transscleral delivery (Chastain et al. 2016).

4.3.3 Light-Activated Systems

Light-activated systems are drugs and delivery systems that are capable of controlled activation. A classic example in the eye is Visudyne®, which is a clinically approved intravenously administered liposomal light-activated system. Visudyne® localizes a photosensitizer in the eye and activates the same by a nonthermal laser light. Visudyne is predominantly used for classic subfoveal choroidal neovascularization in AMD. Laser light at 689 nm is used for activating Visudyne at 15 min after the start of a 10 min infusion with Visudyne. The laser activates verteporfin, a photosensitizer present as an active ingredient in Visudyne. However, photodynamic therapy itself causes neovascularization; thus, the effect of Visudyne® might be insufficient and repeated treatment might be required (Thrimawithana et al. 2011; Christie and Kompella 2008). Photrex® (rostaporfin) is another liposomal photosensitizing system, which did not meet the primary clinical endpoint in wet AMD trials (Huang 2005).

Dendritic porphyrin-loaded micelles are also of potential value in treating choroidal neovascularization. Following laser application micelles accumulate in the neovascularization area and 80% of them remained there up to 7 days (Ideta et al. 2005). Vectosomes or particles made of VP22 protein were also investigated

in vitro and in vivo for light-induced targeted delivery of antisense oligonucleotides. Once injected intravitreally, white light was exposed transsclerally at 24 h to activate vectosomes and release their contents. The results indicated that vectosomes were able to distribute in the various retinal layers and RPE (Normand et al. 2005). Another approach for light-sensitive drug delivery is gold nanoparticle-loaded liposomes, wherein UV light-induced heating of gold nanorods melts and releases the contents of the liposomes (Paasonen et al. 2007). In a recent study, a light-activated in situ gelling system has been designed for suprachoroidal application of bevacizumab, using polycaprolactone dimethacrylate and HEMA. Following 10 min of cross-linking, the gel was able to release bevacizumab for approximately 1 month in a rodent model (Tyagi et al. 2013).

4.3.4 Intraocular Implants for Posterior Segment Drug Delivery

The goal of designing intraocular implants is to provide prolonged and controlled drug delivery up to several months or years using either biodegradable or nonbiodegradable polymers. Sustained-release implants have been studied for chronic diseases that affect the back of the eye such as posterior uveitis, AMD, and diabetic retinopathy. Drug release from these systems occurs either by degradation of the polymer or diffusion through a membrane. Even though some intravitreal implants require surgical implantation, bypassing some drug delivery barriers and reducing dosing frequency and associated side effects are some of their advantages (Jaffe et al. 2006; Guidetti et al. 2008).

Vitrasert® is the first nonbiodegradable, implantable device, designed for ganciclovir delivery and approved by the US FDA in 1996 for the treatment of cytomegalovirus retinitis. Side effects of Vitrasert include endophthalmitis and retinal detachment (Bourges et al. 2006). Retisert®, which is an implant containing fluocinolone acetonide and used for uveitis treatment, was approved by the US FDA in 2005. It is able to release the drug for up to 3 years, but patients who used Retisert® showed a high likelihood of cataract formation and glaucoma (Kempen et al. 2011).

Another FDA-approved intravitreal implant system is Ozurdex®, which is an injectable sustained-release dexamethasone insert approved for posterior uveitis, retinal vein occlusion, and diabetic macular edema. In some cases, it was observed that Ozurdex® implant can migrate into the anterior chamber from its initial location (Khurana et al. 2014; Bratton et al. 2014). Iluvien® is a similarly injectable nonbiodegradable implant system for diabetic macular edema and uveitis treatment, and it was recently approved by the FDA in 2014. It is designed to deliver fluocinolone acetonide for 24–36 months (Pearce et al. 2015).

Neurotech has developed an implant called NT-501 using cell encapsulation technology. Genetically engineered human RPE cells are encapsulated in this implant to secrete ciliary neurotrophic factor. The company is currently conducting clinical trials in patients with early-stage retinitis pigmentosa and Usher syndrome types 2 and 3 (Normand et al. 2005; NeurotechUSA; Lo et al. 2009; Rowe-Rendleman et al. 2014).

4.3.5 Refillable Devices

While the above-described slow-release systems need to be readministered after their intended duration of release, an alternative approach to minimize surgical placement of implants is to use refillable systems and reinject drug as required (Lee et al. 2012). Lo et al. (2009) developed a surgically implantable system to be placed under the conjunctiva and release a specified amount of drug following mechanical activation by a patient's finger (Lo et al. 2009). Since it is a refillable system, it only requires implantation once and allows for continual treatment of chronic diseases. The Replenish MicroPump is also an implantable microreservoir that releases drug at a programmed interval with nanoliter doses, while the drug reservoir can be refilled via transconjunctival injection (Saati et al. 2010). The port delivery system is another refillable device that is in phase II trials for (Pearce et al. 2015) sustained release of ranibizumab in wet AMD patients with subfoveal neovascularization.

4.3.6 Microneedles

Application of microneedles is a recent approach for suprachoroidal and intrascleral drug delivery. There are solid or hollow microneedles available, which were initially developed for transdermal application. Microneedles can deliver free or encapsulated drugs with minimal invasion and may avoid the safety concerns associated with repeated intravitreal applications (Rowe-Rendleman et al. 2014). Based on animal studies, it was reported that insertion site disappears 1 h after microneedle injection (Patel et al. 2012).

Jiang et al. (2009) used human cadaver eyes to test hollow borosilicate microneedle and investigated distribution of sulforhodamine in the posterior segment of the eye (Jiang et al. 2009). The results indicated that distribution was dependent on conditions such as pressure and no significant effect was observed on delivery. Nevertheless, it should be noted that the microneedles were inserted in the sclera and not in the suprachoroidal space.

In another study, Gilger et al. (2013) compared intravitreal drug delivery with suprachoroidal microneedles using triamcinolone acetonide as a model drug using domestic weanling pigs. The delivery system was reported to be safe and effective (Gilger et al. 2013).

Currently, there is one ongoing clinical trial for suprachoroidal drug delivery using microneedles. It is a phase II trial for triamcinolone acetonide suspension in patients with macular edema associated with noninfectious uveitis (NCT02303184).

4.3.7 Transscleral Iontophoresis

Transcorneal iontophoresis has been investigated for anterior segment drug delivery over the years. On the other hand, transscleral iontophoresis is recently gaining attention since it overcomes the lens–iris barrier and delivers drug directly to the back of the eye. In this method an iontophoretic device is placed over the pars plana area on the conjunctiva. A wide variety of drugs have been studied for transscleral iontophoresis including antibiotics, steroids, proteins, genes, drug-containing hydrogels, and nanoparticle delivery systems (Eljarrat-Binstock et al. 2010).

Several studies have shown that transscleral iontophoresis is able to deliver high concentration of drugs to the choroid and retina. Transscleral OcuPhor™ hydrogel has been tested for saline iontophoresis in healthy volunteers. Different intensities have been investigated for 20 and 40 min, and it was reported that the system was well tolerated (Parkinson et al. 2003). DSP-Visulex® is another transscleral iontophoresis system, which consists of a scleral-lens-shaped applicator and currently is in clinical trial phase I/II (Aciont).

Molokhia et al. (2009) reported that transscleral iontophoresis was not efficient in delivering macromolecules to the vitreous in a rabbit model. It was shown that Galbumin, the macromolecule used in the study, was only present in the sclera and conjunctiva (Molokhia et al. 2009). On the other hand, using isolated human sclera showed that large molecules ranging from 51 bp to 2 kbp plasmids can be delivered using iontophoresis (Davies et al. 2003).

In spite of the advantages of transscleral iontophoresis in enhancing drug delivery, tissue damage risk is still a concern. The damage depends on the site of application, duration, and density of the current applied (Eljarrat-Binstock and Domb 2006). Side effects caused by iontophoresis include decrease in endothelial cells, burning, epithelial edema, and inflammation. At high densities choroidal damage and destruction of retinal layers have also been reported (Thrimawithana et al. 2011).

5 Conclusions

Effective drug delivery for the treatment of ocular diseases has always been a challenge especially for the posterior segment, due to the anatomy of the eye, the ocular barriers, and the physiological changes caused by the nature of the diseases. Scientists continue to work on new drug delivery systems to enhance target access, extent of delivery, and duration of drug exposure in order to improve drug efficacy while reducing side effects, in the hope to ultimately improve patient benefit and convenience.

Sustained drug delivery systems, noninvasive approaches for improving back-of-the-eye drug delivery, and contact lenses to prolong ocular surface drug exposure are currently seeing a lot of innovation for improving ocular drug delivery. Despite the promising research, development of eye drops for back-of-the-eye drug effects remains the most formidable challenge. A combination of approaches and a multidisciplinary effort may be needed to overcome this challenge. Translational sciences including understanding of animal models vs. human subjects are critical for improving the predictability of clinical outcomes based on preclinical studies. For translation of ophthalmic drug and gene therapies, it is necessary to create a functional network between scientists, clinicians, regulatory agencies, and industry representatives.

Acknowledgments This was supported in part by the NIH grants EY022097, EY024072, and EY018940 and the US FDA grants FD004929 and FD004719. The authors are thankful to Dr. Ryan A. Kelley for his editorial assistance and review of this chapter.

References

Abdelbary G (2011) Ocular ciprofloxacin hydrochloride mucoadhesive chitosan-coated liposomes. Pharm Dev Technol 16(1):44–56

Aciont. Available from: http://www.aciont.com/technologies/visulex

Agrawal AK, Das M, Jain S (2012) In situ gel systems as 'smart' carriers for sustained ocular drug delivery. Expert Opin Drug Deliv 9(4):383–402

Ahmed I, Patton TF (1985) Importance of the noncorneal absorption route in topical ophthalmic drug delivery. Invest Ophthalmol Vis Sci 26(4):584–587

Aksungur P, Demirbilek M, Denkbas EB, Vandervoort J, Ludwig A, Unlu N (2011) Development and characterization of cyclosporine A loaded nanoparticles for ocular drug delivery: cellular toxicity, uptake, and kinetic studies. J Control Release 151(3):286–294

Amrite AC, Kompella UB (2005) Size-dependent disposition of nanoparticles and microparticles following subconjunctival administration. J Pharm Pharmacol 57(12):1555–1563

Amrite AC, Edelhauser HF, Singh SR, Kompella UB (2008) Effect of circulation on the disposition and ocular tissue distribution of 20 nm nanoparticles after periocular administration. Mol Vis 14:150–160

Ang M (2014) Evaluation of a prednisolone acetate-loaded subconjunctival implant for the treatment of recurrent uveitis in a rabbit model. PLoS One 9(8), e97555

Arakawa Y, Hashida N, Ohguro N, Yamazaki N, Onda M, Matsumoto S et al (2007) Eye-concentrated distribution of dexamethasone carried by sugar-chain modified liposome in experimental autoimmune uveoretinitis mice. Biomed Res 28(6):331–334

Aukunuru JV, Sunkara G, Bandi N, Thoreson WB, Kompella UB (2001) Expression of multidrug resistance-associated protein (MRP) in human retinal pigment epithelial cells and its interaction with BAPSG, a novel aldose reductase inhibitor. Pharm Res 18(5):565–572

Badawi AA, El-Laithy HM, El Qidra RK, El Mofty H, El dally M (2008) Chitosan based nanocarriers for indomethacin ocular delivery. Arch Pharm Res 31(8):1040–1049

Barar J, Javadzadeh AR, Omidi Y (2008) Ocular novel drug delivery: impacts of membranes and barriers. Expert Opin Drug Deliv 5(5):567–581

Baydoun L, Muller-Goymann CC (2003) Influence of n-octenylsuccinate starch on in vitro permeation of sodium diclofenac across excised porcine cornea in comparison to Voltaren ophtha. Eur J Pharm Biopharm 56(1):73–79

Bhagav P, Upadhyay H, Chandran S (2011) Brimonidine tartrate-eudragit long-acting nanoparticles: formulation, optimization, in vitro and in vivo evaluation. AAPS PharmSciTech 12(4):1087–1101

Bikbova G, Bikbov M (2014) Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin. Acta Ophthalmol 92(1):e30–e34

Bock F, Matthaei M, Reinhard T, Bohringer D, Christoph J, Ganslandt T et al (2014) High-dose subconjunctival cyclosporine A implants do not affect corneal neovascularization after high-risk keratoplasty. Ophthalmology 121(9):1677–1682

Bourges JL, Bloquel C, Thomas A, Froussart F, Bochot A, Azan F et al (2006) Intraocular implants for extended drug delivery: therapeutic applications. Adv Drug Deliv Rev 58(11):1182–1202

Bourkiza R, Lee V (2012) A review of the complications of lacrimal occlusion with punctal and canalicular plugs. Orbit 31(2):86–93

Bratton ML, He YG, Weakley DR (2014) Dexamethasone intravitreal implant (Ozurdex) for the treatment of pediatric uveitis. J AAPOS 18(2):110–113

Calvo P, Alonso MJ, Vila-Jato JL, Robinson JR (1996) Improved ocular bioavailability of indomethacin by novel ocular drug carriers. J Pharm Pharmacol 48(11):1147–1152

Carmen AL, Fernando Y, Rafael BI, Angel C (2006) Imprinted soft contact lenses as norfloxacin delivery systems. J Control Release 113:236–424
Chaiyasan W, Srinivas SP, Tiyaboonchai W (2013) Mucoadhesive chitosan-dextran sulfate nanoparticles for sustained drug delivery to the ocular surface. J Ocul Pharmacol Ther 29 (2):200–207
Chang SC, Bundgaard H, Buur A, Lee VH (1987) Improved corneal penetration of timolol by prodrugs as a means to reduce systemic drug load. Invest Ophthalmol Vis Sci 28(3):487–491
Chastain JE, Sanders ME, Curtis MA, Chemuturi NV, Gadd ME, Kapin MA et al (2016) Distribution of topical ocular nepafenac and its active metabolite amfenac to the posterior segment of the eye. Exp Eye Res 145:58–67
Chauhan A (2015) Ocular drug delivery role of contact lenses. Allied Ophthal Sci 26(2):131–135
Chee SP (2012) Moxifloxacin punctum plug for sustained drug delivery. J Ocul Pharmacol Ther 28 (4):340–349
Cheruvu NP, Kompella UB (2006) Bovine and porcine transscleral solute transport: influence of lipophilicity and the Choroid-Bruch's layer. Invest Ophthalmol Vis Sci 47(10):4513–4522
Cheruvu NP, Amrite AC, Kompella UB (2008) Effect of eye pigmentation on transscleral drug delivery. Invest Ophthalmol Vis Sci 49(1):333–341
Chien DS, Tang-Liu DD, Woodward DF (1997) Ocular penetration and bioconversion of prostaglandin F2alpha prodrugs in rabbit cornea and conjunctiva. J Pharm Sci 86(10):1180–1186
Chopra P, Hao J, Li SK (2010) Iontophoretic transport of charged macromolecules across human sclera. Int J Pharm 388(1–2):107–113
Christie JG, Kompella UB (2008) Ophthalmic light sensitive nanocarrier systems. Drug Discov Today 13(3–4):124–134
Civiale C, Bucaria F, Piazza S, Peri O, Miano F, Enea V (2004) Ocular permeability screening of dexamethasone esters through combined cellular and tissue systems. J Ocul Pharmacol Ther 20 (1):75–84
Civiale C, Licciardi M, Cavallaro G, Giammona G, Mazzone MG (2009) Polyhydroxyethylaspartamide-based micelles for ocular drug delivery. Int J Pharm 378 (1–2):177–186
Cohen AE, Assang C, Patane MA, From S, Korenfeld M (2012) Avion study investigators. Evaluation of dexamethasone phosphate delivered by ocular iontophoresis for treating noninfectious anterior uveitis. Ophthalmology 119(1):66–73
Contreras-Ruiz L, Zorzi GK, Hileeto D, Lopez-Garcia A, Calonge M, Seijo B et al (2013) A nanomedicine to treat ocular surface inflammation: performance on an experimental dry eye murine model. Gene Ther 20(5):467–477
Cruysberg LP, Nuijts RM, Geroski DH, Koole LH, Hendrikse F, Edelhauser HF (2002) In vitro human scleral permeability of fluorescein, dexamethasone-fluorescein, methotrexate-fluorescein and rhodamine 6G and the use of a coated coil as a new drug delivery system. J Ocul Pharmacol Ther 18(6):559–569
Davies NM (2000) Biopharmaceutical considerations in topical ocular drug delivery. Clin Exp Pharmacol Physiol 27(7):558–562
Davies JB, Ciavatta VT, Boatright JH, Nickerson JM (2003) Delivery of several forms of DNA, DNA-RNA hybrids, and dyes across human sclera by electrical fields. Mol Vis 9 (68–69):569–578
Davis BM, Normando EM, Guo L, Turner LA, Nizari S, O'Shea P et al (2014) Topical delivery of Avastin to the posterior segment of the eye in vivo using annexin A5-associated liposomes. Small 10(8):1575–1584
De Campos AM, Sanchez A, Alonso MJ (2001) Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. Int J Pharm 224(1–2):159–168
de Paiva CS, Schwartz CE, Gjorstrup P, Pflugfelder SC (2012) Resolvin E1 (RX-10001) reduces corneal epithelial barrier disruption and protects against goblet cell loss in a murine model of dry eye. Cornea 31(11):1299–1303

Dey S, Anand BS, Patel J, Mitra AK (2003) Transporters/receptors in the anterior chamber: pathways to explore ocular drug delivery strategies. Expert Opin Biol Ther 3(1):23–44
Di Tommaso C, Torriglia A, Furrer P, Behar-Cohen F, Gurny R, Moller M (2011) Ocular biocompatibility of novel cyclosporin A formulations based on methoxy poly(ethylene glycol)-hexylsubstituted poly(lactide) micelle carriers. Int J Pharm 416(2):515–524
Dong Y, Dong P, Huang D, Mei L, Xia Y, Wang Z et al (2015) Fabrication and characterization of silk fibroin-coated liposomes for ocular drug delivery. Eur J Pharm Biopharm 91:82–90
Dunlevy JR, Rada JA (2004) Interaction of lumican with aggrecan in the aging human sclera. Invest Ophthalmol Vis Sci 45(11):3849–3856
Durairaj C, Shah JC, Senapati S, Kompella UB (2009a) Prediction of vitreal half-life based on drug physicochemical properties: quantitative structure-pharmacokinetic relationships (QSPKR). Pharm Res 26(5):1236–1260
Durairaj C, Kim SJ, Edelhauser HF, Shah JC, Kompella UB (2009b) Influence of dosage form on the intravitreal pharmacokinetics of diclofenac. Invest Ophthalmol Vis Sci 50(10):4887–4897
Durairaj C, Kadam RS, Chandler JW, Hutcherson SL, Kompella UB (2010) Nanosized dendritic polyguanidilyated translocators for enhanced solubility, permeability, and delivery of gatifloxacin. Invest Ophthalmol Vis Sci 51(11):5804–5816
Duvvuri S, Majumdar S, Mitra AK (2003) Drug delivery to the retina: challenges and opportunities. Expert Opin Biol Ther 3(1):45–56
Eljarrat-Binstock E, Domb AJ (2006) Iontophoresis: a non-invasive ocular drug delivery. J Control Release 110(3):479–489
Eljarrat-Binstock E, Pe'er J, Domb AJ (2010) New techniques for drug delivery to the posterior eye segment. Pharm Res 27(4):530–543
Eperon S, Bossy-Nobs L, Petropoulos IK, Gurny R, Guex-Crosier Y (2008) A biodegradable drug delivery system for the treatment of postoperative inflammation. Int J Pharm 352 (1–2):240–247
Eytan GD, Kuchel PW (1999) Mechanism of action of P-glycoprotein in relation to passive membrane permeation. Int Rev Cytol 190:175–250
Fujisawa T, Miyai H, Hironaka K, Tsukamoto T, Tahara K, Tozuka Y et al (2012) Liposomal diclofenac eye drop formulations targeting the retina: formulation stability improvement using surface modification of liposomes. Int J Pharm 436(1–2):564–567
Furrer E, Berdugo M, Stella C, Behar-Cohen F, Gurny R, Feige U et al (2009) Pharmacokinetics and posterior segment biodistribution of ESBA105, an anti-TNF-alpha single-chain antibody, upon topical administration to the rabbit eye. Invest Ophthalmol Vis Sci 50(2):771–778
Gan L, Gan Y, Zhu C, Zhang X, Zhu J (2009) Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine A: in vitro and in vivo results. Int J Pharm 365(1–2):143–149
Gan L, Han S, Shen J, Zhu J, Zhu C, Zhang X et al (2010) Self-assembled liquid crystalline nanoparticles as a novel ophthalmic delivery system for dexamethasone: improving preocular retention and ocular bioavailability. Int J Pharm 396(1–2):179–187
Gaudana R, Jwala J, Boddu SHS, Mitra AK (2009) Recent perspectives in ocular drug delivery. Pharm Res 26(5):1197–1216
Gaudana R, Ananthula HK, Parenky A, Mitra AK (2010) Ocular drug delivery. AAPS J 12 (3):348–360
Ghate D, Edelhauser HF (2006) Ocular drug delivery. Expert Opin Drug Deliv 3(2):275–287
Ghazi NG, Abboud EB, Nowilaty SR, Alkuraya H, Alhommadi A, Cai H et al (2016) Treatment of retinitis pigmentosa due to MERTK mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum Genet 135(3):327–343
Gilger BC, Abarca EM, Salmon JH, Patel S (2013) Treatment of acute posterior uveitis in a porcine model by injection of triamcinolone acetonide into the suprachoroidal space using microneedles. Invest Ophthalmol Vis Sci 54(4):2483–2492
Gu JM, Robinson JR, Leung SH (1988) Binding of acrylic polymers to mucin/epithelial surfaces: structure-property relationships. Crit Rev Ther Drug Carrier Syst 5(1):21–67

Guidetti B, Azema J, Malet-Martino M, Martino R (2008) Delivery systems for the treatment of proliferative vitreoretinopathy: materials, devices and colloidal carriers. Curr Drug Deliv 5 (1):7–19

Gupta C, Chauhan A (2011) Ophthalmic delivery of cyclosporine A by punctal plugs. J Control Release 150(1):70–76

Gupta AK, Madan S, Majumdar DK, Maitra A (2000) Ketorolac entrapped in polymeric micelles: preparation, characterisation and ocular anti-inflammatory studies. Int J Pharm 209(1–2):1–14

Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A, Mittal G (2011) Biodegradable levofloxacin nanoparticles for sustained ocular drug delivery. J Drug Target 19(6):409–417

Habib FS, Fouad EA, Abdel-Rhaman MS, Fathalla D (2010) Liposomes as an ocular delivery system of fluconazole: in-vitro studies. Acta Ophthalmol 88(8):901–904

Halhal M, Renard G, Courtois Y, BenEzra D, Behar-Cohen F (2004) Iontophoresis: from the lab to the bed side. Exp Eye Res 78:751–757

Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L et al (2008) Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. Hum Gene Ther 19 (10):979–990

Hillman JS (1974) Management of acute glaucoma with pilocarpine-soaked hydrophilic lens. Br J Ophthalmol 58(7):674–679

Hobden JA, Reidy JJ, O'Callaghan RJ, Insler MS, Hill JM (1990) Ciprofloxacin iontophoresis for aminoglycoside-resistant pseudomonal keratitis. Invest Ophthalmol Vis Sci 31(10):1940–1944

Holden CA, Tyagi P, Thakur A, Kadam R, Jadhav G, Kompella UB et al (2012) Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs. Nanomed Nanotechnol Biol Med 8(5):776–783

Horvat G, Budai-Szucs M, Berko S, Szabo-Revesz P, Soos J, Facsko A et al (2015) Comparative study of nanosized cross-linked sodium-, linear sodium- and zinc-hyaluronate as potential ocular mucoadhesive drug delivery systems. Int J Pharm 494(1):321–328

Hosoya K, Lee VH (1997) Cidofovir transport in the pigmented rabbit conjunctiva. Curr Eye Res 16(7):693–697

Hosoya K, Tachikawa M (2012) The inner blood-retinal barrier: molecular structure and transport biology. Adv Exp Med Biol 763:85–104

Hosoya K, Horibe Y, Kim KJ, Lee VH (1997) Na(+)-dependent L-arginine transport in the pigmented rabbit conjunctiva. Exp Eye Res 65(4):547–553

Hosoya K, Lee VH, Kim KJ (2005) Roles of the conjunctiva in ocular drug delivery: a review of conjunctival transport mechanisms and their regulation. Eur J Pharm Biopharm 60(2):227–240

Hosoya K, Tomi M, Tachikawa M (2011) Strategies for therapy of retinal diseases using systemic drug delivery: relevance of transporters at the blood-retinal barrier. Expert Opin Drug Deliv 8 (12):1571–1587

Hsu KH, Carbia BE, Plummer C, Chauhan A (2015) Dual drug delivery from vitamin E loaded contact lenses for glaucoma therapy. Eur J Pharm Biopharm 94:312–321

Hu FQ, Li YH, Yuan H, Zeng S (2006) Novel self-aggregates of chitosan oligosaccharide grafted stearic acid: preparation, characterization and protein association. Pharmazie 61(3):194–198

Huang Z (2005) A review of progress in clinical photodynamic therapy. Technol Cancer Res Treat 4(3):283–293

Hughes PM, Mitra AK (1993) Effect of acylation on the ocular disposition of acyclovir. II: corneal permeability and anti-HSV 1 activity of 2′-esters in rabbit epithelial keratitis. J Ocul Pharmacol 9(4):299–309

Hughes PM, Olejnik O, Chang-Lin JE, Wilson CG (2005) Topical and systemic drug delivery to the posterior segments. Adv Drug Deliv Rev 57(14):2010–2032

Hui A, Sheardown H, Jones L (2012) Acetic and acrylic acid molecular imprinted model silicone hydrogel materials for ciprofloxacin-hcl delivery. Materials 5(1):81–107

Ibrahim HK, El-Leithy IS, Makky AA (2010) Mucoadhesive nanoparticles as carrier systems for prolonged ocular delivery of gatifloxacin/prednisolone bitherapy. Mol Pharm 7(2):576–585

Ideta R, Tasaka F, Jang WD, Nishiyama N, Zhang GD, Harada A et al (2005) Nanotechnology-based photodynamic therapy for neovascular disease using a supramolecular nanocarrier loaded with a dendritic photosensitizer. Nano Lett 5(12):2426–2431

Jackson TL, Antcliff R, Hillenkamp J, Marshall J (2003) Human retinal molecular weight exclusion limit and estimate of species variation. Invest Ophthalmol Vis Sci 44(5):2141–2146

Jaffe GJ, Martin D, Callanan D, Pearson PA, Levy B, Comstock T et al (2006) Fluocinolone acetonide implant (Retisert) for noninfectious posterior uveitis - thirty-four-week results of a multicenter randomized clinical study. Ophthalmology 113(6):1020–1027

Jain-Vakkalagadda B, Dey S, Pal D, Mitra AK (2003) Identification and functional characterization of a Na+-independent large neutral amino acid transporter, LAT1, in human and rabbit cornea. Invest Ophthalmol Vis Sci 44(7):2919–2927

Jain-Vakkalagadda B, Pal D, Gunda S, Nashed Y, Ganapathy V, Mitra AK (2004) Identification of a Na+-dependent cationic and neutral amino acid transporter, B(0,+), in human and rabbit cornea. Mol Pharm 1(5):338–346

Janoria KG, Hariharan S, Paturi D, Pal D, Mitra AK (2006) Biotin uptake by rabbit corneal epithelial cells: role of sodium-dependent multivitamin transporter (SMVT). Curr Eye Res 31 (10):797–809

Jiang J, Moore JS, Edelhauser HF, Prausnitz MR (2009) Intrascleral drug delivery to the eye using hollow microneedles. Pharm Res 26(2):395–403

Jung HJ, Chauhan A (2012) Temperature sensitive contact lenses for triggered ophthalmic drug delivery. Biomaterials 33(7):2289–2300

Kambhampati SP, Mishra MK, Mastorakos P, Oh Y, Lutty GA, Kannan RM (2015) Intracellular delivery of dendrimer triamcinolone acetonide conjugates into microglial and human retinal pigment epithelial cells. Eur J Pharm Biopharm 95(Pt B):239–249

Kang SJ, Durairaj C, Kompella UB, O'Brien JM, Grossniklaus HE (2009) Subconjunctival nanoparticle carboplatin in the treatment of murine retinoblastoma. Arch Ophthalmol 127 (8):1043–1047

Karlgard CC, Wong NS, Jones LW, Moresoli C (2003) In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. Int J Pharm 257(1–2):141–151

Karn PR, Kim HD, Kang H, Sun BK, Jin SE, Hwang SJ (2014) Supercritical fluid-mediated liposomes containing cyclosporin A for the treatment of dry eye syndrome in a rabbit model: comparative study with the conventional cyclosporin A emulsion. Int J Nanomedicine 9:3791–3800

Kassem MA, Abdel Rahman AA, Ghorab MM, Ahmed MB, Khalil RM (2007) Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. Int J Pharm 340(1–2):126–133

Katiyar S, Pandit J, Mondal RS, Mishra AK, Chuttani K, Aqil M et al (2014) In situ gelling dorzolamide loaded chitosan nanoparticles for the treatment of glaucoma. Carbohydr Polym 102:117–124

Katragadda S, Talluri RS, Mitra AK (2006) Modulation of P-glycoprotein-mediated efflux by prodrug derivatization: an approach involving peptide transporter-mediated influx across rabbit cornea. J Ocul Pharmacol Ther 22(2):110–120

Kaur IP, Smitha R (2002) Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. Drug Dev Ind Pharm 28(4):353–369

Kempen JH, Altaweel MM, Holbrook JT, Jabs DA, Louis TA, Sugar EA et al (2011) Randomized comparison of systemic anti-inflammatory therapy versus fluocinolone acetonide implant for intermediate, posterior, and panuveitis: the multicenter uveitis steroid treatment trial. Ophthalmology 118(10):1916–1926

Khan W, Aldouby YH, Avramoff A, Domb AJ (2012) Cyclosporin nanosphere formulation for ophthalmic administration. Int J Pharm 437(1–2):275–276

Khurana RN, Appa SN, McCannel CA, Elman MJ, Wittenberg SE, Parks DJ et al (2014) Dexamethasone implant anterior chamber migration risk factors, complications, and management strategies. Ophthalmology 121(1):67–71

Kim SH, Lutz RJ, Wang NS, Robinson MR (2007) Transport barriers in transscleral drug delivery for retinal diseases. Ophthalmic Res 39(5):244–254
Kirchhof S, Goepferich AM, Brandl FP (2015) Hydrogels in ophthalmic applications. Eur J Pharm Biopharm 95(Pt B):227–238
Klang S, Abdulrazik M, Benita S (2000) Influence of emulsion droplet surface charge on indomethacin ocular tissue distribution. Pharm Dev Technol 5(4):521–532
Kompella UB, Kadam RS, Lee VH (2010) Recent advances in ophthalmic drug delivery. Ther Deliv 1(3):435–456
Kompella UB, Amrite AC, Pacha Ravi R, Durazo SA (2013) Nanomedicines for back of the eye drug delivery, gene delivery, and imaging. Prog Retin Eye Res 36:172–198
Konat Zorzi G, Contreras-Ruiz L, Parraga JE, Lopez-Garcia A, Romero Bello R, Diebold Y et al (2011) Expression of MUC5AC in ocular surface epithelial cells using cationized gelatin nanoparticles. Mol Pharm 8(5):1783–1788
Kuno N, Fujii S (2011) Recent advances in ocular drug delivery systems. Polymers (Basel) 3 (1):193–221
Kuwano M, Ibuki H, Morikawa N, Ota A, Kawashima Y (2002) Cyclosporine A formulation affects its ocular distribution in rabbits. Pharm Res 19(1):108–111
Lallemand F, Furrer P, Felt-Baeyens O, Gex-Fabry M, Dumont JM, Besseghir K et al (2005) A novel water-soluble cyclosporine A prodrug: ocular tolerance and in vivo kinetics. Int J Pharm 295(1–2):7–14
Lallemand F, Varesio E, Felt-Baeyens O, Bossy L, Hopfgartner G, Gurny R (2007) Biological conversion of a water-soluble prodrug of cyclosporine A. Eur J Pharm Biopharm 67 (2):555–561
Law SL, Huang KJ, Chiang CH (2000) Acyclovir-containing liposomes for potential ocular delivery. Corneal penetration and absorption. J Control Release 63(1–2):135–140
Lee VH (1983) Esterase activities in adult rabbit eyes. J Pharm Sci 72(3):239–244
Lee VH, Robinson JR (1986) Topical ocular drug delivery: recent developments and future challenges. J Ocul Pharmacol 2(1):67–108
Lee JH, Pidaparti RM, Atkinson GM, Moorthy RS (2012) Design of an implantable device for ocular drug delivery. J Drug Deliv 2012:527516
Li N, Zhuang C, Wang M, Sun X, Nie S, Pan W (2009) Liposome coated with low molecular weight chitosan and its potential use in ocular drug delivery. Int J Pharm 379(1):131–138
Lo R, Li PY, Saati S, Agrawal RN, Humayun MS, Meng E (2009) A passive MEMS drug delivery pump for treatment of ocular diseases. Biomed Microdevices 11(5):959–970
Losa C, Calvo P, Castro E, Vila-Jato JL, Alonso MJ (1991) Improvement of ocular penetration of amikacin sulphate by association to poly(butylcyanoacrylate) nanoparticles. J Pharm Pharmacol 43(8):548–552
Losa C, Marchal-Heussler L, Orallo F, Vila Jato JL, Alonso MJ (1993) Design of new formulations for topical ocular administration: polymeric nanocapsules containing metipranolol. Pharm Res 10(1):80–87
Lu C, Yoganathan RB, Kociolek M, Allen C (2013) Hydrogel containing silica shell cross-linked micelles for ocular drug delivery. J Pharm Sci 102(2):627–637
Luo Q, Zhao J, Zhang X, Pan W (2011) Nanostructured lipid carrier (NLC) coated with Chitosan Oligosaccharides and its potential use in ocular drug delivery system. Int J Pharm 403 (1–2):185–191
Mac Gabhann F, Demetriades AM, Deering T, Packer JD, Shah SM, Duh E et al (2007) Protein transport to choroid and retina following periocular injection: theoretical and experimental study. Ann Biomed Eng 35(4):615–630
Macha S, Mitra AK (2003) Overview of ocular drug delivery. In: Mitra AK (ed) Ophthalmic drug delivery systems, 2nd edn. Marcel Dekker, New York, pp 1–12
Mainolfi N, Powers J, Amin J, Long D, Lee W, McLaughlin ME et al (2013) An effective prodrug strategy to selectively enhance ocular exposure of a cannabinoid receptor (CB1/2) agonist. J Med Chem 56(13):5464–5472

Maiti S, Paul S, Mondol R, Ray S, Sa B (2011) Nanovesicular formulation of brimonidine tartrate for the management of glaucoma: in vitro and in vivo evaluation. AAPS PharmSciTech 12 (2):755–763
Majumdar S, Nashed YE, Patel K, Jain R, Itahashi M, Neumann DM et al (2005) Dipeptide monoester ganciclovir prodrugs for treating HSV-1-induced corneal epithelial and stromal keratitis: in vitro and in vivo evaluations. J Ocul Pharmacol Ther 21(6):463–474
Mannermaa E, Vellonen KS, Urtti A (2006) Drug transport in corneal epithelium and blood-retina barrier: emerging role of transporters in ocular pharmacokinetics. Adv Drug Deliv Rev 58 (11):1136–1163
Matsuo T, Masuda I, Yasuda T, Matsuo N (1996) Gene transfer to the retina of rat by liposome eye drops. Biochem Biophys Res Commun 219(3):947–950
Mehanna MM, Elmaradny HA, Samaha MW (2010) Mucoadhesive liposomes as ocular delivery system: physical, microbiological, and in vivo assessment. Drug Dev Ind Pharm 36 (1):108–118
Mishima S (1981) Clinical pharmacokinetics of the eye. Proctor lecture. Invest Ophthalmol Vis Sci 21(4):504–541
Mitra AK, Velagaleti PR, Grau UM (2010) Topical drug delivery systems for ophthalmic use. Google Patents
Mohammadi S, Jones L, Gorbet M (2014) Extended latanoprost release from commercial contact lenses: in vitro studies using corneal models. PLoS One 9(9), e106653
Molokhia SA, Jeong EK, Higuchi WI, Li SK (2008) Examination of barriers and barrier alteration in transscleral iontophoresis. J Pharm Sci 97(2):831–844
Molokhia SA, Jeong EK, Higuchi WI, Li SK (2009) Transscleral iontophoretic and intravitreal delivery of a macromolecule: study of ocular distribution in vivo and postmortem with MRI. Exp Eye Res 88(3):418–425
Molokhia SA, Sant H, Simonis J, Bishop CJ, Burr RM, Gale BK et al (2010) The capsule drug device: novel approach for drug delivery to the eye. Vision Res 50(7):680–685
Molokhia SA, Thomas SC, Garff KJ, Mandell KJ, Wirostko BM (2013) Anterior eye segment drug delivery systems: current treatments and future challenges. J Ocul Pharmacol Ther 29 (2):92–105
Monem AS, Ali FM, Ismail MW (2000) Prolonged effect of liposomes encapsulating pilocarpine HCl in normal and glaucomatous rabbits. Int J Pharm 198(1):29–38
Moosa RM, Choonara YE, du Toit LC, Kumar P, Carmichael T, Tomar LK et al (2014) A review of topically administered mini-tablets for drug delivery to the anterior segment of the eye. J Pharm Pharmacol 66(4):490–506
Muchtar S, Almog S, Torracca MT, Saettone MF, Benita S (1992) A submicron emulsion as ocular vehicle for delta-8-tetrahydrocannabinol: effect on intraocular pressure in rabbits. Ophthalmic Res 24(3):142–149
Nagarsenker MS, Londhe VY, Nadkarni GD (1999) Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery. Int J Pharm 190(1):63–71
Nair KL, Vidyanand S, James J, Kumar GSV (2012) Pilocarpine-loaded poly(DLlactic-co-glycolic acid) nanoparticles as potential candidates for controlled drug delivery with enhanced ocular pharmacological response. J Appl Polym Sci 124:2030–2036
NeurotechUSA. Available from: http://www.neurotechusa.com/cntfrenexus.html
Nicoli S, Ferrari G, Quarta M, Macaluso C, Santi P (2009) In vitro transscleral iontophoresis of high molecular weight neutral compounds. Eur J Pharm Sci 36(4–5):486–492
Normand N, Valamanesh F, Savoldelli M, Mascarelli F, BenEzra D, Courtois Y et al (2005) VP22 light controlled delivery of oligonucleotides to ocular cells in vitro and in vivo. Mol Vis 11 (21):184–191
Olsen TW, Aaberg SY, Geroski DH, Edelhauser HF (1998) Human sclera: thickness and surface area. Am J Ophthalmol 125(2):237–241

Paasonen L, Laaksonen T, Johans C, Yliperttula M, Kontturi K, Urth A (2007) Gold nanoparticles enable selective light-induced contents release from liposomes. J Control Release 122 (1):86–93

Parkinson TM, Ferguson E, Febbraro S, Bakhtyari A, King M, Mundasad M (2003) Tolerance of ocular iontophoresis in healthy volunteers. J Ocul Pharmacol Ther 19(2):145–151

Pascolini D, Mariotti SP (2012) Global estimates of visual impairment: 2010. Br J Ophthalmol 96 (5):614–618

Patel SR, Berezovsky DE, McCarey BE, Zarnitsyn V, Edelhauser HF, Prausnitz MR (2012) Targeted administration into the suprachoroidal space using a microneedle for drug delivery to the posterior segment of the eye. Invest Ophthalmol Vis Sci 53(8):4433–4441

Pearce W, Hsu J, Yeh S (2015) Advances in drug delivery to the posterior segment. Curr Opin Ophthalmol 26(3):233–239

Pederson J (2006) Fluid physiology of the subretinal space. In: Rayan SJ (ed) Retina, 4th edn. Elsevier Inc., Philadelphia, pp 1909–1920

Pehlivan SB, Yavuz B, Calamak S, Ulubayram K, Kaffashi A, Vural I et al (2015) Preparation and in vitro/in vivo evaluation of cyclosporin A-loaded nanodecorated ocular implants for subconjunctival application. J Pharm Sci 104(5):1709–1720

Peng CC, Burke MT, Carbia BE, Plummer C, Chauhan A (2012) Extended drug delivery by contact lenses for glaucoma therapy. J Control Release 162(1):152–158

Pepic I, Jalsenjak N, Jalsenjak I (2004) Micellar solutions of triblock copolymer surfactants with pilocarpine. Int J Pharm 272(1–2):57–64

Pepic I, Hafner A, Lovric J, Pirkic B, Filipovic-Grcic J (2010) A nonionic surfactant/chitosan micelle system in an innovative eye drop formulation. J Pharm Sci 99(10):4317–4325

Peyman GA, Schulman JA, Khoobehi B, Alkan HM, Tawakol ME, Mani H (1989) Toxicity and clearance of a combination of liposome-encapsulated ganciclovir and trifluridine. Retina 9 (3):232–236

Phan CM, Subbaraman L, Jones L (2014) Contact lenses for antifungal ocular drug delivery: a review. Expert Opin Drug Deliv 11(4):537–546

Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G (2002) Eudragit RS100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen. Eur J Pharm Sci 16 (1–2):53–61

Prabhu P, Nitish KR, Koland M, Harish N, Vijayanarayan K, Dhondge G et al (2010) Preparation and evaluation of nano-vesicles of brimonidine tartrate as an ocular drug delivery system. J Young Pharm 2(4):356–361

Quintana-Hau JD, Cruz-Olmos E, Lopez-Sanchez MI, Sanchez-Castellanos V, Baiza-Duran L, Gonzalez JR et al (2005) Characterization of the novel ophthalmic drug carrier Sophisen in two of its derivatives: 3A Ofteno and Modusik-A Ofteno. Drug Dev Ind Pharm 31(3):263–269

Rafie F, Javadzadeh Y, Javadzadeh AR, Ghavidel LA, Jafari B, Moogooee M et al (2010) In vivo evaluation of novel nanoparticles containing dexamethasone for ocular drug delivery on rabbit eye. Curr Eye Res 35(12):1081–1089

Raghava S, Hammond M, Kompella UB (2004) Periocular routes for retinal drug delivery. Expert Opin Drug Deliv 1(1):99–114

Ranta VP, Mannermaa E, Lummepuro K, Subrizi A, Laukkanen A, Antopolsky M et al (2010) Barrier analysis of periocular drug delivery to the posterior segment. J Control Release 148 (1):42–48

Reimondez-Troitino S, Csaba N, Alonso MJ, de la Fuente M (2015) Nanotherapies for the treatment of ocular diseases. Eur J Pharm Biopharm 95(Pt B):279–293

Reimondez-Troitino S, Csaba N, Alonso MJ, de la Fuente M (2015b) Nanotherapies for the treatment of ocular diseases. Eur J Pharm Biopharm 95:279–293

Rodriguez-Aller M, Kaufmann B, Guillarme D, Stella C, Furrer P, Rudaz S et al (2012) In vivo characterisation of a novel water-soluble cyclosporine A prodrug for the treatment of dry eye disease. Eur J Pharm Biopharm 80(3):544–552

Rowe-Rendleman CL, Durazo SA, Kompella UB, Rittenhouse KD, Di Polo A, Weiner AL et al (2014) Drug and gene delivery to the back of the eye: from bench to bedside. Invest Ophthalmol Vis Sci 55(4):2714–2730

Ruponen M, Urtti A (2015) Undefined role of mucus as a barrier in ocular drug delivery. Eur J Pharm Biopharm 96:442–446

Saati S, Lo R, Li PY, Meng E, Varma R, Humayun MS (2010) Mini drug pump for ophthalmic use. Curr Eye Res 35(3):192–201

Sachinkumar P, Atul K, Sandip B, Shitalkumar P (2015) Formuation and evaluation of an in situ gel for ocular drug delivery of anticonjunctival drug. Cellul Chem Technol 49(1):35–40

Saha P, Yang JJ, Lee VH (1998) Existence of a p-glycoprotein drug efflux pump in cultured rabbit conjunctival epithelial cells. Invest Ophthalmol Vis Sci 39(7):1221–1226

Sanders DR, Goldstick B, Kraff C, Hutchins R, Bernstein MS, Evans MA (1983) Aqueous penetration of oral and topical indomethacin in humans. Arch Ophthalmol 101(10):1614–1616

Santipharp P, Laman LA (2008) Ophthalmic nanoparticulate formulation of a cyclooxygenase-2 selective inhibitor. Patent US20080145430 A1

Schoenwald RD (1990) Ocular drug delivery. Pharmacokinetic considerations. Clin Pharmacokinet 18(4):255–269

Sedlacek J (1965) Possibility of the application of ophthalmic drugs with the use of gel contact lenses. Cesk Oftalmol 21(6):509–512

Shell JW (1985) Ophthalmic drug delivery systems. Drug Dev Res 6(3):245–261

Shen Y, Tu J (2007) Preparation and ocular pharmacokinetics of ganciclovir liposomes. AAPS J 9 (3):E371–E377

Shen J, Deng Y, Jin X, Ping Q, Su Z, Li L (2010) Thiolated nanostructured lipid carriers as a potential ocular drug delivery system for cyclosporine A: improving in vivo ocular distribution. Int J Pharm 402(1–2):248–253

Shen J, Gan L, Zhu C, Zhang X, Dong Y, Jiang M et al (2011) Novel NSAIDs ophthalmic formulation: flurbiprofen axetil emulsion with low irritancy and improved anti-inflammation effect. Int J Pharm 412(1-2):115–122

Shukla A, Fuller RC, Hammond PT (2011) Design of multi-drug release coatings targeting infection and inflammation. J Control Release 155(2):159–166

Sigurdsson HH, Kirch J, Lehr CM (2013) Mucus as a barrier to lipophilic drugs. Int J Pharm 453 (1):56–64

Sjoquist B, Stjernschantz J (2002) Ocular and systemic pharmacokinetics of latanoprost in humans. Surv Ophthalmol 47(Suppl 1):S6–S12

Spataro G, Malecaze F, Turrin CO, Soler V, Duhayon C, Elena PP et al (2010) Designing dendrimers for ocular drug delivery. Eur J Med Chem 45(1):326–334

Spraul CW, Lang GE, Grossniklaus HE, Lang GK (1999) Histologic and morphometric analysis of the choroid, Bruch's membrane, and retinal pigment epithelium in postmortem eyes with age-related macular degeneration and histologic examination of surgically excised choroidal neovascular membranes. Surv Ophthalmol 44(Suppl 1):S10–S32

Steuer H, Jaworski A, Elger B, Kaussmann M, Keldenich J, Schneider H et al (2005) Functional characterization and comparison of the outer blood-retina barrier and the blood-brain barrier. Invest Ophthalmol Vis Sci 46(3):1047–1053

Stratford RE Jr, Lee VH (1985) Ocular aminopeptidase activity and distribution in the albino rabbit. Curr Eye Res 4(9):995–999

Sznitowska M, Zurowska-Pryczkowska K, Janicki S, Jarvinen T (1999) Miotic effect and irritation potential of pilocarpine prodrug incorporated into a submicron emulsion vehicle. Int J Pharm 184(1):115–120

Taban M, Chen B, Perry JD (2006) Update on punctal plugs. Compr Ophthalmol Updat 7 (5):205–212, discussion 213–214

Talluri RS, Katragadda S, Pal D, Mitra AK (2006) Mechanism of L-ascorbic acid uptake by rabbit corneal epithelial cells: evidence for the involvement of sodium-dependent vitamin C transporter 2. Curr Eye Res 31(6):481–489

Thrimawithana TR, Young S, Bunt CR, Green C, Alany RG (2011) Drug delivery to the posterior segment of the eye. Drug Discov Today 16(5-6):270–277
Ticho U, Blumenthal M, Zonis S, Gal A, Blank I, Mazor ZW (1979) A clinical trial with Piloplex--a new long-acting pilocarpine compound: preliminary report. Ann Ophthalmol 11(4):555–561
Tirucherai GS, Dias C, Mitra AK (2002) Corneal permeation of ganciclovir: mechanism of ganciclovir permeation enhancement by acyl ester prodrug design. J Ocul Pharmacol Ther 18(6):535–548
Tong YC, Chang SF, Liu CY, Kao WW, Huang CH, Liaw J (2007) Eye drop delivery of nano-polymeric micelle formulated genes with cornea-specific promoters. J Gene Med 9 (11):956–966
Trivedi R, Kompella UB (2010) Nanomicellar formulations for sustained drug delivery: strategies and underlying principles. Nanomedicine (Lond) 5(3):485–505
Trivedi R, Redente EF, Thakur A, Riches DW, Kompella UB (2012) Local delivery of biodegradable pirfenidone nanoparticles ameliorates bleomycin-induced pulmonary fibrosis in mice. Nanotechnology 23(50):505101
Tyagi P, Barros M, Stansbury JW, Kompella UB (2013) Light-activated, in situ forming gel for sustained suprachoroidal delivery of bevacizumab. Mol Pharm 10(8):2858–2867
Vadlapudi AD, Vadlapatla RK, Kwatra D, Earla R, Samanta SK, Pal D et al (2012a) Targeted lipid based drug conjugates: a novel strategy for drug delivery. Int J Pharm 434(1-2):315–324
Vadlapudi AD, Vadlapatla RK, Mitra AK (2012b) Current and emerging antivirals for the treatment of cytomegalovirus (CMV) retinitis: an update on recent patents. Recent Pat Antiinfect Drug Discov 7(1):8–18
Vadlapudi AD, Cholkar K, Vadlapatla RK, Mitra AK (2014) Aqueous nanomicellar formulation for topical delivery of biotinylated lipid prodrug of acyclovir: formulation development and ocular biocompatibility. J Ocul Pharmacol Ther 30(1):49–58
Valls R, Vega E, Garcia ML, Egea MA, Valls JO (2008) Transcorneal permeation in a corneal device of non-steroidal anti-inflammatory drugs in drug delivery systems. Open Med Chem J 2:66–71
Vandamme TF, Brobeck L (2005) Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. J Control Release 102(1):23–38
Vega E, Egea MA, Valls O, Espina M, Garcia ML (2006) Flurbiprofen loaded biodegradable nanoparticles for ophthalmic administration. J Pharm Sci 95(11):2393–2405
Vinogradov SV, Batrakova EV, Kabanov AV (2004) Nanogels for oligonucleotide delivery to the brain. Bioconjug Chem 15(1):50–60
Vooturi SK, Kadam RS, Kompella UB (2012) Transporter targeted gatifloxacin prodrugs: synthesis, permeability, and topical ocular delivery. Mol Pharm 9(11):3136–3146
Wadhwa S, Paliwal R, Paliwal SR, Vyas SP (2010) Hyaluronic acid modified chitosan nanoparticles for effective management of glaucoma: development, characterization, and evaluation. J Drug Target 18(4):292–302
Wong VG (1989) Biodegradable ocular implants. Google Patents
Wu J, Zhang JJ, Koppel H, Jacob TJ (1996) P-glycoprotein regulates a volume-activated chloride current in bovine non-pigmented ciliary epithelial cells. J Physiol 491(Pt 3):743–755
Xiang CD, Batugo M, Gale DC, Zhang T, Ye J, Li C et al (2009) Characterization of human corneal epithelial cell model as a surrogate for corneal permeability assessment: metabolism and transport. Drug Metab Dispos 37(5):992–998
Xu X, Weng YH, Xu L, Chen H (2013) Sustained release of avastin (R) from polysaccharides cross-linked hydrogels for ocular drug delivery. Int J Biol Macromol 60:272–276
Yavuz B, Pehlivan SB, Vural I, Unlu N (2015) In vitro/in vivo evaluation of Dexamethasone--PAMAM dendrimer complexes for retinal drug delivery. J Pharm Sci 104(11):3814–3823
Yellepeddi VK, Sheshala R, McMillan H, Gujral C, Jones D, Raghu Raj Singh T (2015) Punctal plug: a medical device to treat dry eye syndrome and for sustained drug delivery to the eye. Drug Discov Today 20(7):884–889

Yenice I, Mocan MC, Palaska E, Bochot A, Bilensoy E, Vural I et al (2008) Hyaluronic acid coated poly-epsilon-caprolactone nanospheres deliver high concentrations of cyclosporine A into the cornea. Exp Eye Res 87(3):162–167

Yu J, Xu X, Yao FL, Luo ZC, Jin L, Xie BB et al (2014) In situ covalently cross-linked PEG hydrogel for ocular drug delivery applications. Int J Pharm 470(1–2):151–157

Yuan XB, Yuan YB, Jiang W, Liu J, Tian EJ, Shun HM et al (2008) Preparation of rapamycin-loaded chitosan/PLA nanoparticles for immunosuppression in corneal transplantation. Int J Pharm 349(1–2):241–248

Zhang T, Xiang CD, Gale D, Carreiro S, Wu EY, Zhang EY (2008) Drug transporter and cytochrome P450 mRNA expression in human ocular barriers: implications for ocular drug disposition. Drug Metab Dispos 36(7):1300–1307

Ocular Pharmacology of Tear Film, Dry Eye, and Allergic Conjunctivitis

Shilpa Gulati and Sandeep Jain

Contents

Abstract

Dry Eye Disease (DED) is "a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear-film instability with potential damage to the ocular surface." DED comprises two

S. Gulati • S. Jain (✉)
Department of Ophthalmology and Visual Sciences, Illinois Eye and Ear Infirmary, College of Medicine, University of Illinois at Chicago, 1855 West Taylor Street, Chicago, IL, USA
e-mail: shgulati@gmail.com; jains@uic.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_73

etiologic categories: aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE). Diagnostic workup of DED should include clinical history, symptom questionnaire, fluorescein TBUT, ocular surface staining grading, Schirmer I/II, lid and meibomian pathology, meibomian expression, followed by other available tests. New diagnostic tests employ the Oculus Keratograph, which performs non-invasive tear-film analysis and a bulbar redness (BR). The TearLab Osmolarity Test enables rapid clinical evaluation of tear osmolarity. Lipiview is a recently developed diagnostic tool that uses interferometry to quantitatively evaluate tear-film thickness. In DED, epithelial and inflammatory cells produce a variety of inflammatory mediators. A stagnant tear film and decreased concentration of mucin result in the accumulation of inflammatory factors that can penetrate tight junctions and cause epithelial cell death. DED treatment algorithms are based on severity of clinical signs and symptoms, and disease etiology. Therapeutic approaches include lubricating artificial tears and immunomodulatory agents.

Keywords
Conjunctivitis • Diagnostics • Dry eye • Ocular surface • Tear film • Therapy

1 Tear Film Structure and Physiology

The tear film forms a layer approximately 3 μm thick and 3 μL in volume on the anterior conjunctival surface, and serves multiple functions including lubrication, antimicrobial protection, nutrition, maintenance of corneal transparency and surface stem cell population, removal of debris, and preservation of the quality of image projected to the retina. Estimates of tear turnover rate range between 0.12 and 1.47 μL/min (5–22.2%/min) (King-Smith et al. 2000; Dartt and Willcox 2013).

Tear film composition is dynamic, responding to environmental conditions in order to maintain ocular surface homeostasis. The film itself is an emulsion of three components: an outer lipid layer secreted by the meibomian, Zeis, and Moll glands; an intermediate aqueous layer secreted by the main and accessory lacrimal glands; and an inner mucin layer secreted by conjunctival goblet cells. The lipid layer is composed of a combination of low polarity lipids, such as wax and cholesterol esters, and high polarity lipids, such as triglycerides, free fatty acids, and phospholipids. The aqueous layer is composed of inorganic salts, bicarbonate ions, glucose, urea, enzymes, proteins, and glycoproteins. While traditionally understood as three separate and distinct layers, new studies suggest that the mucin and aqueous layers integrate to create a gradient of decreasing mucin concentration outwards to the aqueous layer (Dartt and Willcox 2013).

2 Dry Eye Disease

Dry eye disease (DED) is a complex symptomatic syndrome with myriad clinical variations, defined by the International Dry Eye WorkShop (DEWS) as "a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear-film instability with potential damage to the ocular surface." (International Dry Eye Workshop (DEWS) Definition and Classification 2007) DED, synonymous with keratoconjunctivitis sicca (KCS), was subdivided by DEWS into two etiologic categories: aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE).

The pathophysiology of DED involves numerous pathways leading to a final common denominator of lacrimal functional unit (LFU) dysfunction. The LFU consists of the ocular surface (cornea, limbus, conjunctiva, conjunctival blood vessels), tears and their associated machinery (lacrimal glands, meibomian glands, goblet cells, epithelial cells, nasolacrimal duct), and relevant components of the nervous, endocrine, immune, and vascular systems. These elements preserve corneal clarity by maintaining lubrication, nutrition, and the surface stem cell population, while minimizing inflammation and microbial overgrowth.

2.1 Aqueous-Deficient Dry Eye

ADDE is caused by reduced lacrimal tear secretion, and can be further divided into two subgroups: Sjogren syndrome (SSDE) and non-Sjogren syndrome (non-SS) conditions. Sjogren syndrome is an autoimmune exocrinopathy in which activated T lymphocytes infiltrate lacrimal and salivary glands, causing apoptosis of acinar and ductular cells and subsequent dysfunction. Dry eye caused by gland hyposecretion is further worsened by a neurosecretory block, which may be caused by antibodies directed against muscarinic receptors of the glands, or inflammatory cytokines in tear film. Clinically, patients present with symptoms of both dry eye and dry mouth (xerostomia); diagnosis can be aided by lab tests for autoantigens that are expressed by surface epithelial cells (anti-Ro and anti-La). Sjogren syndrome may occur as primary disease, but more often is secondary to a known autoimmune condition, most commonly systemic lupus erythematosus (SLE), polyarteritis nodosa, granulomatosis with polyangiomatosis, systemic sclerosis, primary biliary cirrhosis, or mixed connective tissue disease.

Non-SS dry eye can be divided into four categories of conditions: primary lacrimal gland deficiencies, secondary lacrimal gland deficiencies, obstruction of the lacrimal gland ducts, and reflex hyposecretion. Primary lacrimal gland deficiency is most commonly attributable to age-related dry eye (ARDE). As normal individuals age, glands are obstructed by the accumulation of ductal changes, including periductal fibrosis, interacinar fibrosis, paraductal blood vessel loss, and acinar cell atrophy. Other uncommon forms of primary lacrimal gland deficiency are: congenital alacrima, a rare cause of childhood DED; and familial dysautonomia (Riley Day syndrome), a progressive, autosomal recessive, neuronal

developmental abnormality characterized by insensitivity to pain. In the latter condition, impaired sympathetic and parasympathetic lacrimal gland innervation and poor ocular surface sensation impede both emotional and reflex tearing (International Dry Eye Workshop (DEWS) Definition and Classification 2007).

Secondary lacrimal gland deficiencies may be associated with a number of systemic conditions in which the lacrimal gland is infiltrated by cells causing dysfunction: sarcoidosis (invasion by non-caseating granulomas); lymphoma (lymphomatous tissue); and AIDS (CD-8T lymphocytes). In graft vs. host disease (GVHD), fibrosis occurs ~6 months after transplantation with the invasion of periductal CD-4 and CD-8T cells, and antigen-presenting fibroblasts. Ablation or denervation of the lacrimal gland secondary to trauma or surgery may also cause DED.

Cicatrizing disorders that lead to lacrimal gland duct obstruction include: trachoma, which causes trichiasis, tarsal and conjunctival scarring, and meibomian gland dysfunction; cicatricial or mucous membrane pemphigoid, which causes severe conjunctival blistering; erythema multiforme, which is an acute and self-limited cutaneous disorder of variable etiology (drug, infection, malignancy) that may cause conjunctival scarring; Stevens–Johnson syndrome; and chemical and thermal burns.

Finally, any impairment of reflex hyposecretion can cause non-SS ADDE. Physiologic tearing occurs in response to a variety of stimuli: the cornea and lid margins are densely innervated by sensory branches of the trigeminal nerve, lacrimal and meibomian glands receive both parasympathetic and sympathetic innervation, and goblet cells have parasympathetic innervation. These pathways form the reflex arcs that control reflex tear secretion. However, surface sensory loss may lead to decreased reflex hyposecretion and blink rate (which causes dry eye through evaporative tear loss). Impaired corneal sensitivity is found in a multitude of common conditions including chronic contact lens wear, diabetes, refractive surgery, or neurotrophic keratitis (caused by HSV or HZV infection, or CN V damage); it can also occur secondary to systemic beta blockers, atropine, keratoplasty, or the limbal incision of extracapsular cataract surgery. Reflex motor block, or damage to CN VII, also leads to reflex hyposecretion since damage to postganglionic, parasympathetic fibers to the lacrimal gland decreases secretomotor function, and lagophthalmos due to incomplete lid closure increases evaporative loss of tears. Trauma may cause damage to these pathways, as well as systemic medications including antihistamines, beta blockers, antispasmodics, diuretics, tricyclic antidepressants, and selective serotonin reuptake inhibitors.

2.2 Evaporative Dry Eye

EDE is characterized by a pathologically high level of tear evaporation and can be caused by internal conditions that affect lid structures or dynamics, or environmental factors and exposures. An example of an intrinsic cause is the reduced blink rate that accompanies driving, watching TV, reading, and computer work, leading to rapid evaporation. In contrast, environmental factors act directly on the external

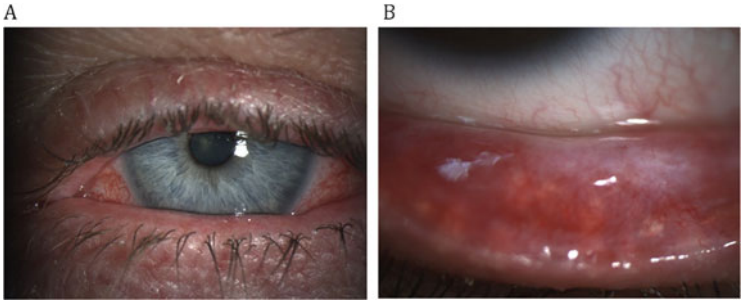

Fig. 1 (**a**) Lid margin telangiectasias, madarosis, and margin thickening and irregularity are characteristic of inflammatory conditions such as ocular rosacea. (**b**) Chronic severe inflammation can lead to conjunctival fibrosis

surface; common culprits include central heating, dry climate, air pollution, wind, chemical burns, and contact lens wear.

The most common cause of EDE is meibomian gland dysfunction (MGD), which is chronic inflammation of the eyelid margin posterior to the gray line that may be accompanied by squamous debris, terminal gland obstruction, and qualitative or quantitative changes in glandular secretion. MGD can be identified at slit lamp by morphologic features of duct orifice plugging, increased viscosity of excreta, or inability to express oil from the glands (Fig. 1). It can be evaluated with qualitative grading, meibography to measure the degree of gland dropout, or meibometry to quantify the amount of oil in the lid margin reservoir. Causes may be local (posterior blepharitis); systemic (such as acne rosacea, seborrhoeic dermatitis, atopic dermatitis); or syndromal (anhidrotic ectodermal dysplasia, ectrodactyly syndrome, Turner syndrome). Cicatricial MGD may occur secondary to local tissue damage such as with trauma, burn, pemphigoid, erythema multiforme, or vernal keratoconjunctivitis. Other causes of MGD include meibomian gland replacement, which occurs in distichiasis; gland deficiency, which may be congenital or acquired; or reversible gland atrophy, which is caused by isotretinoin acne treatment.

Intrinsic EDE causes include conditions that compromise lid apposition or decrease blink rate. For example, dry eye is common and often severe in thyroid eye disease, which causes lid retraction and proptosis leading to an increased palpebral fissure and lagophthalmos. The decline in blink rate that may accompany Parkinson's disease results from a decrease in the quantity of dopaminergic neurons in the substantia nigra, and is proportional to disease severity. In addition to increased tear evaporation time, infrequent blinking impairs clearance of lipid-contaminated mucin.

Tear film instability may be caused by indoor environmental factors, such as high temperature and low relative humidity (RH), as found in air-conditioned cars, offices, and airplane cabins. "Cool and dry" conditions are ideal, with recommended RH of about 40%. Likewise, outdoor exposure to sun, dust, and wind has

been shown to worsen DED (Gayton 2009). These environmental exposures and trauma may also lead to corneal abnormalities, such as pterygium, which can disrupt the tear film and lead to symptoms of dry eye.

While women overall are more likely to have dry eye symptoms, hormonal studies suggest that sex hormone changes influence ocular surface conditions through both aqueous production and evaporative mechanisms: by impacting tear secretion, meibomian gland function, and conjunctival goblet cell density. This relationship is not fully understood but, in clinical studies, women taking oral contraceptives have been found to have significantly higher goblet cell density. Chronic reduction in androgen levels has been associated with meibomian gland dysfunction, the absence of anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β), and the release of proinflammatory cytokines such as interleukins (IL-1β, IL-2), interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α). Accordingly, DED is more common in low-androgen conditions: postmenopause, primary ovarian failure, and autoimmune conditions. Furthermore, postmenopausal women on hormone-replacement therapy have a higher prevalence of DED, especially in women who are on estrogen only regimens (Gayton 2009; Peters and Colby 2013).

3 Epidemiology

Prevalence of dry eye increases with age, affecting 10% of adults age 30–60 and 15% of adults over age 65, and is more common among females (Weisenthal et al. 2015; Schaumberg et al. 2003). Based on data from large population based studies, the Women's Health Study (WHS) and the Physicians' Health Study (PHS), an estimated 3.23 million women and 1.68 million men in America over the age of 50 suffer from DED (International Dry Eye Workshop (DEWS) Epidemiology 2007).

The epidemiology of DED is limited by the different definitions employed by various studies. However, consistent evidence has been found to implicate several risk factors, including female sex, older age, postmenopausal estrogen therapy, a diet that low in omega 3 essential fatty acids, a diet with a high ratio of omega 6 to omega 3 fatty acids, antihistamines, connective tissue disease, history of refractive surgery, vitamin A deficiency, androgen deficiency, hepatitis C infection, radiation therapy, and bone marrow transplantation (International Dry Eye Workshop (DEWS) Epidemiology 2007).

4 Symptom Analysis

Patients with DED present with a diversity of symptoms that include pain, dryness, grittiness, itching, redness, burning or stinging, foreign body sensation, and light sensitivity. As symptoms may persist or worsen over time, DED has been shown to negatively impact patients' quality of life, both in general and vision-related. Given

the variability of clinical tests, assessing DED symptoms in their entirety becomes fundamentally important to guide treatment decisions.

Hallek et al. at the University of Illinois at Chicago developed a four-domain symptom burden tool for comprehensive clinical evaluation of DED impact. Symptoms are classified into two main dimensions, sensory and reactive, and further subdivided into four domains: the sensory dimension is divided into symptom persistence and symptom intensity, and the reactive dimension is divided into activity interference and affective interference. A combination of visual analog, numerical, verbal descriptive, and verbal rating scales were then employed to calculate a numeric score for a patient's experience (Hallak et al. 2013).

In a cross-sectional 48-patient pilot study of this symptom burden assessment tool, the authors found that persistence of symptoms, and not intensity, was correlated with affective interference (or the "mood" of individuals). Because DED has been shown to correlate with anxiety and depression, the study concluded the need for an affective component to be added to standardized DED questionnaires, such as the Ocular Surface Disease Index (OSDI) (Li et al. 2011; Galor et al. 2012; Fernandez et al. 2013).

Authors also found that irrespective of clinical signs, the majority of patients reporting low symptom intensity received less aggressive treatments; management is governed by perceived severity. However, there is a well-established disconnect between signs and symptoms of DED (Mertzanis et al. 2005; Nichols et al. 2004; Johnson 2009). Traditional therapies for DED replace or conserve a patient's tears without correcting the underlying disease process. As a result the study concluded that clinicians need to objectively assess the type and severity of DED in order to effectively address the disease pathophysiology.

5 Diagnosis

Diagnostic tests to assess tear stability, ocular staining, and reflex tear flow, should be chosen based on patients' report of symptoms. Per DEWS the recommended order of tests is as follows: Clinical history, symptom questionnaire, fluorescein TBUT, ocular surface staining grading, Schirmer I/II, lid and meibomian pathology, meibomian expression, followed by other available tests. The DEWS Diagnostic Methodology Subcommittee recommends the administration of structured symptomatology questionnaires to patients presenting with potential DED in order to use clinic time most efficiently. Several questionnaires have been validated and clinicians may choose one based on practical factors such as time, staff available to implement, and end use (International Dry Eye Workshop (DEWS) 2007).

Tear turnover may be evaluated by measuring tearfilm breakup time (TFBUT) in seconds. A standard amount of fluorescein is applied to the eye (as a drop, or by placing a fluorescein-impregnated strip that is wet with saline) initial instruction for the patient to blink in order to distribute the fluorescein. The patient should then be asked to open the eyes without blinking. Viewing under cobalt blue or yellow barrier light at the slit lamp, the clinician measures TFBUT: the interval between

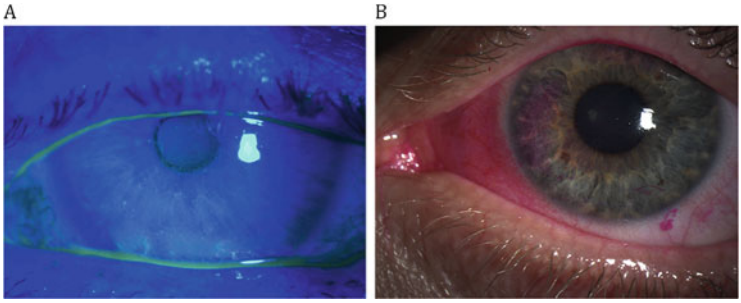

Fig. 2 (**a**) Sodium fluorescein dye stains devitalized epithelial cells, and highlights a narrow tear lake. (**b**) Rose bengal dye stains devitalized epithelial cells, those unprotected by mucin or glycocalyx, and proliferating cells; however, it carries the disadvantage of ocular surface toxicity

the last complete blink and the appearance of micelle, or disruption in the tear film. TFBUT cut-off for dry eye diagnosis is less than 10 s. While this test does not require precision to identify extreme cases, it is subject to operator error; dye must be instilled delicately so that it doesn't elicit reflex tearing, and a standard amount of fluorescein should be placed in the eye (International Dry Eye Workshop (DEWS) 2007).

Epitheliopathy is a characteristic feature of DED, and surface integrity is quantified by grading of ocular surface staining with vital dyes. Most commonly used is hydrophilic sodium fluorescein dye, which diffuses into the corneal stroma to highlight areas of epithelial loss when viewed under cobalt blue light. In contrast, lissamine green (LG) adheres to epithelial cells that are devitalized or unprotected by mucin or glycocalyx; rose bengal (RB) adheres to these in addition to proliferating cells (Fig. 2). LG and RB dyes bear a number of advantages: both are poorly visible within the tear film so the dye in the tear film does not obscure the staining pattern (as with fluorescein, Fig. 2); and since these dyes do not diffuse into the substantia propria of the conjunctiva, there staining pattern lasts longer. While both are well visualized with the backdrop of a light colored iris, they are difficult to see against a darkly pigmented background. RB staining also carries the disadvantage of ocular toxicity, causing stinging and pain that are worse with photoactivation. The degree of staining is dose dependent, however, so instilling a smaller amount or concentration of dye will modify the result. Therefore this test is best performed after instillation of topical anesthetic, and should be followed with saline irrigation (International Dry Eye Workshop (DEWS) 2007).

For each ocular surface staining test, a saline moistened dye-impregnated strip is first used to instill dye on the inferior palpebral conjunctiva. After 15 s, corneal and conjunctival staining are graded by a slit lamp examination (cobalt blue filter is used for fluorescein dye, and rose filter is used for RB and LG). The 1995 National Eye Institute/Industry Workshop scale divides the cornea into 5 zones and the conjunctiva into 6 zones, and each zone is graded from 0 to 3 based on the density of punctate staining. The final staining score is the sum of the individual scores

from all 11 zones. While a greatly simplified Oxford system has since been developed to evaluate ocular surface staining, the NEI scale is preferable because it isolates the visual axis in its own corneal zone (Lemp 1995).

Aqueous tear deficiency is best assessed with the Schirmer test, in which standardized Schirmer strips are bent at the notch and placed carefully over the lower lid margin near the temporal angle of the lids. Strips remain in place for 5 min while the patient keeps both eyes closed, and afterwards the wetting length is measured. The Schirmer I test may be conducted with or without the application of topical anesthetic; the diagnostic cut-off for severe dry eye is generally considered 5 mm or less tear production. Schirmer II is preceded by stimulation of nasal mucosa. Intrasubject variation invalidates comparison of results between individual patients, but same subject comparison can prove valuable despite day-to-day variation of results for a given patient (Whitcher et al. 2010).

6 New Diagnostics

A number of new diagnostic tests employ the Oculus Keratograph, which performs non-invasive tear film analysis (Fig. 3). The keratograph uses a Placido bowl with a camera aperture that has a fixation mark in the center. The device provides consistent illumination, allowing scanning of the exposed bulbar conjunctiva. The system generates a bulbar redness (BR) score automatically, which is based on the area percentage ratio between the vessels and the rest of the analyzed area. The BR range between 0.0 (0%) and 4.0 (40%, the maximum ratio) objectively evaluates ocular surface redness.

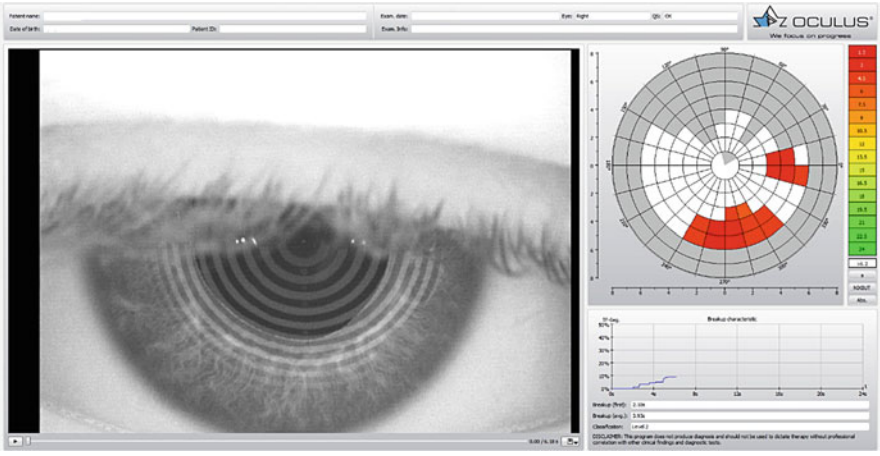

Fig. 3 The Oculus Keratograph calculates first TFBUT (time at first break up of tears) and average TFBUT (average time of all breakup incidents), as well as tear meniscus height (TMH) throughout the cornea

The same machine is also used to calculate non-invasive keratograph tear film breakup time (NIKBUT); this is a more objective measure of tear film stability than a slit lamp evaluation of TFBUT, and does not require application of fluorescein. The keratograph measures tear breakup time twice for each eye using infrared (IR) video and automatically generates two measures of output: NIKBUT-first (time at first break up of tears) and NIKBUT-average (average time of all breakup incidents).

IR images are also used to evaluate tear meniscus height (TMH), an element of tear film quality. The Oculus TMH tool uses an integrated ruler to measure TMH, which is graded perpendicular to the lid margin at the central point relative to the pupil center.

The failure of lacrimal tear secretion involved in ADDE causes tear film hyperosmolarity, and subsequent epithelial cell hyperosmolarity, which in turn initiates a cascade of inflammatory events involving MAP kinases, NFkB signaling pathways, cytokines IL-1 and TNF-alpha, and matrix metalloproteinase 9 (MMP-9, an endopeptidase involved in tissue remodeling). The presence of tear hyperosmolarity and MMP-9 in tear film are therefore valuable tools as they implicate an aqueous deficient etiology of DED (though it is not possible to differentiate between dysfunction of the lacrimal gland itself or other elements of the tear-production pathway).

The TearLab Osmolarity Test, FDA approved in 2009, enables rapid clinical evaluation of tear osmolarity. An abnormal salinity reflects a failure of homeostatic regulation, a key feature of DED; when left unchecked, hyperosmolar tears in early stage DED will lead to damage of the cornea and conjunctiva characteristic of late stage disease. The outcome is continuous: the higher the osmolarity, the more severe the dry eye. To perform the test, a Test Card is touched to the inferior tear meniscus to collect ~50 nL of tear fluid by passive capillary action. The machine then utilizes a temperature-corrected impedance measurement to provide an indirect assessment of osmolarity. One prospective clinical study evaluated the relationship between clinical metrics of DED (OSDI, TFBUT, surface staining, Schirmer, meibomian scoring, tear osmolarity) with a composite of these scores; tear osmolarity was the only marker to demonstrate a linear relationship without significant scatter. This test is also benefitted by its objectivity, quantitative nature, and operator independence (Sullivan et al. 2010).

Lipiview is a recently developed diagnostic tool that uses interferometry to quantitatively evaluate tear film thickness (Fig. 4). Infrared and transillumination images created through dynamic illumination and adaptive transillumination allow clinicians to visualize eyelid morphology and detect structural changes suggesting gland dilation, atrophy, or drop out in severe disease (Hosaka et al. 2011).

Inflammatory markers in tears are also a new focus of diagnostic tests. For example, corneal endothelial cells produce endopeptidases, such as MMP-9, after desiccating stress; this promotes corneal extracellular matrix degradation and epithelial cell loss. InflammaDry is an FDA-approved clinical tool that measures MMP-9 protein in human tears. The test must be performed prior to instilling ocular anesthetic or performing Schirmer testing. Tear fluid sample is collected by dabbing the sampling fleece on the inside of the patient's palpebral conjunctiva

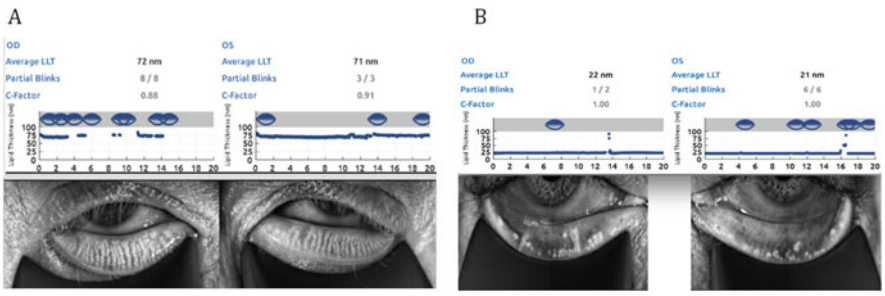

Fig. 4 Lipiview is a new diagnostic tool that uses dynamic infrared transillumination to visualize structural changes suggesting meibomian gland dilation, atrophy, or dropout. Images (**a**) and (**b**) demonstrate a visible contrast between normal gland structure and gland dropout, and a dramatic difference in lipid layer thickness (LLT)

at least 6–8 times, and then rest against the conjunctiva for 5 s. Once this sample is immersed in a buffer solution for at least 20 s, the test cassette is laid flat for 10 min. The result, represented by indicator lines, is binary: a positive result reflects a concentration of MMP-9 $>=40$ ng/mL, and a negative result reflects a concentration of MMP-9 <40 ng/mL.

7 Pathophysiology

7.1 The Innate and Adaptive Immune Systems

The innate immune system is the first line of defense in preventing microorganism invasion of the ocular surface. This system is composed of several nonspecific mechanical and chemical elements, including epithelial tight junctions and epithelial cell sloughing, reflex tearing, the barrier of closed eyelids, the conjunctival mucous membrane, mucins (glycosylated proteins produced by epithelial cells), anti-inflammatory factors (such as lactoferrin), proteolytic enzymes, pattern recognition receptors (PRPs), toll like receptors (TLRs), antimicrobial peptides (such as lysozyme, defensins, cathelicidins, and lipocalin), secreted phospholipase A2 (sPLA2), and secretory Immunoglobulin A (sIgA).

In the case of LFU dysfunction, epithelial and inflammatory cells produce a variety of inflammatory mediators that suppress T cell activation and inhibit complement-mediated tissue damage. Blinking, tear secretion, and tear drainage are all essential to flush away these inflammatory mediators from the ocular surface.

When they accumulate on the ocular surface, the proinflammatory cytokines IL-1 and IFN-γ cause squamous metaplasia of epithelial cells; IFN-γ inhibits goblet cell differentiation; intrinsic (stress-associated mitogen-activated protein kinase) and extrinsic (TNF and Fas/Fas ligand) pathways cause apoptosis of epithelial cells; and MMPs (such as MMP-9) promote corneal extracellular matrix degradation (Stevenson et al. 2012). Therefore, in the context of a stagnant tear film and

decreased concentration of mucin, the resultant accumulation of inflammatory factors can penetrate tight junctions and cause epithelial cell death (Narayanan et al. 2013).

Once these primary protective mechanisms are infiltrated, the adaptive immune system is activated. Adaptive immunity is acquired through specific antigen exposures and is later triggered through re-exposure. Antigen-presenting cells (APCs), such as dendritic cells, elicit a response of T lymphocytes and antibody-producing B lymphocytes, to attack a recognized pathogen.

For example, exposure of corneal epithelial cells to elevated tear osmolarity activates apoptosis of the epithelial surface cells and stress-associated mitogen-activated protein kinases. These in turn stimulate transcription factors (such as nuclear factor kB and activator protein 1) and the production of proinflammatory cytokines, chemokines, and MMPs. Cytokines and chemokines facilitate the maturation of APCs, which migrate to lymphoid tissue to expand the population of CD4+ helper T cell subtypes 1 and 17 (TH1 and TH17). These T cells travel to the ocular surface where TH 1 secretes IFN γ, and TH 17 secretes IL 17, which stimulates the production of MMPs. IFN γ and MMP-9 cause further damage to epithelial cells as noted above. The key to this proinflammatory cycle is the snowball effect (Stevenson et al. 2012).

7.2 External Stressors and Hyperosmolarity

Factors that disturb the homeostasis of the LFU ecosystem increase tear osmolarity. In a healthy state, the osmolarity of tear film is 296–302 mOsm/L; however, in patients with DED, this value rises to 316–360 mOsm/L. The hyperosmolar environment is caused by aqueous tear deficiency and/or increased evaporation of tears, and it stimulates a cascade of osmotic, mechanical, and inflammatory damage, as described above. It also stimulates formation of neutrophil extracellular traps (NET). Numerous neutrophils egress from circulation into tear film during ocular surface inflammation, and NETS on the ocular surface of patients with severe tear deficiency are associated with expression of type I interferon, plus inflammatory cytokines like interleukin-6 and tumor necrosis factor-alpha in ocular surface cells.

Tibrewal et al. recently reported that the amount of NETs released by neutrophils increased exponentially as hyperosmolarity increased, suggesting that NETs likely play a larger role in severe DED with greater hyperosmolarity (>350 mOsm/L) than mild dry eye with minimally elevated osmolarity. Furthermore, neutrophils were found to continue to release NETs, albeit in reduced amounts, even if the iso-osmolar milieu was restored (Tibrewal et al. 2014). The clinical implication of this finding is that although pulsed application of iso-osmolar or hypotonic artificial tear eye drops will intermittently reduce osmolarity, neutrophils will continue to release NETs once exposure to hyperosmolarity recurs.

8 Management

The development of pharmacological therapies for DED has been limited by our incomplete understanding of the mechanism, pathogenesis, and clinical manifestation of DED. Classification of DED by etiology is valuable in choosing a therapeutic approach because while ADDE and EDE often coexist and most treatments are effective for both types (such as artificial tears, cyclosporine, and steroid drops), some therapies are harmful if inappropriately used. For example, punctual plugs therapeutically increase tear retention time in ADDE, but in the presence of MGD they also increase ocular surface exposure to toxic inflammatory factors (Whitcher et al. 2010).

8.1 Stepwise Treatment

The International Task Force at Delphi in 2006 developed stepwise treatment algorithms based on severity of clinical signs and symptoms, and disease etiology. This was modified by the International DEWS in 2007, which published a dry eye grading scheme that assigns a severity score of 1–4+ based on each of 9 diagnostic metrics: discomfort, severity and frequency; visual symptoms; conjunctival injection; conjunctival staining; corneal staining (severity/location); corneal/tear signs; lid/meibomian glands; TFBUT; and Schirmer score.

The DEWS treatment scheme is based on severity. For level 1 it recommends education and counseling, environmental management, elimination of offending systemic medications, and preserved tear substitutes or allergy eye drops. If these are inadequate, level 2 treatment involves preservative-free tears, gels and ointments, steroids, cyclosporine A, secretagogues such as pilocarpine (now rarely used); and nutritional supplements. Level 3 treatment entails tetracycline, autologous serum tears, and punctal plugs (after control of inflammation). For refractory level 4 DED, they recommended topical vitamin A, contact lenses, acetylcysteine, and moisture goggles, or surgical treatment (such as tarsorrhaphy) (International Dry Eye Workshop (DEWS) Management and Therapy 2007).

Prior to pharmacologic therapies, clinicians should consider risk factor modification, such as: smoking cessation, home humidifier use, diet modification to increase consumption of omega three fatty acids, and discontinuation of systemic medications associated with dry eye (diuretics, antihistamines, anticholinergics, and psychotropics are most common).

8.2 Therapeutic Approaches

Lubricating artificial tears are hypotonic or isotonic buffered solutions, of neutral to slightly alkaline pH, containing electrolytes (including potassium to maintain corneal thickness, and bicarbonate to promote recovery of epithelial barrier function), a high colloidal osmolality (such as in glycerin or erythritol, which counteract

hyperosmolar tear film), and viscosity agents to enhance retention time. Viscosity in drop and ointment formulations is achieved with macromolecular complexes: short-acting preparations are often based on carboxymethyl cellulose, polyvinyl alcohol, polyethylene glycol, or hydroxymethyl cellulose; longer-acting ointments are based on carbomer gels or paraffin. Lubricant agents are distributed both over-the-counter and by prescription; though there have been no large, randomized controlled clinical trials to compare the many ocular lubricants on the market, this class of agents remains the mainstay of DED therapy.

While multi-dose artificial tears are mandated by the FDA to contain preservatives such as benzalkonium chloride (BAK) in order to inhibit microbial growth, these are toxic in high quantities (instillation more than 4–6 times/day). The cytotoxic damage they inflict on epithelial cell shape, junctions, and microvilli can cause epithelial cell necrosis; the effect increases with decreased tear secretion and turnover (as found in DED), high concentration of preservatives, and frequency of exposure. For more severe dry eye characterized by lacrimal hyposecretion requiring frequent administration of lubricants, punctual occlusion, or use of multiple drops that have preservative elements, preservative-free preparations in single-use vials are preferable.

Tears may also be substituted with biologically compatible drops, autologous serum tears, which are made from serum that is isolated from the cellular components of blood. To prepare, peripheral blood (20 mL) is taken from a patient, centrifuged to separate the serum, which is then diluted to 20% with sterile saline. The tears are stored in a bottle coated with protection from UV light and must be stored in a freezer for 1 month only, to maintain the desired composition. Serum has an osmotic pressure (300 mOsm) and pH (7.2–7.5) nearly matching that of natural tears (302 mOsm and 7.4, respectively). While the exact mechanism of action is unknown, serum tears contain key ingredients of epidermal growth factor (EGF), vitamin A, TGF-beta, and fibronectin, which promote epithelial healing and are also found in natural tears (Tsubota and Yamada 1992). Similarly, serum has been shown to upregulate mucin production, and contains serum antiprotease which inhibits collagenases. The addition of these trophic components to the water and electrolytes found in traditional artificial tears has been demonstrated to effectively treat DED and persistent epithelial defects (PEDs), and improve TFBUT and vital dye staining when compared to artificial tears.

Immunomodulatory agents also play a significant therapeutic role for DED. The most commonly used anti-inflammatory drop is topical cyclosporine A (CsA) 0.05%, as it has been shown to alleviate the symptoms of DED in about 50% of patients. CsA is a lipophilic peptide that binds with a group of proteins known as cyclophilins. By binding with cyclophilin A, which is found in the cytosol, CsA inhibits calcineurin, a protein phosphatase that dephosphorylates regulatory sites on transcription factors such as nuclear factor of activated T-lymphocytes (NFATs). Through this mechanism CsA selectively inhibits interleukin-2 (IL-2), which is required for the transcription of T cells, thereby suppressing a cell-mediated immune response and interrupting inflammatory cytokine production. However, since the T cell life span can last 110–180 days, CsA may take several months to

take effect and a short course of topical steroids may be prescribed at the outset of treatment. CsA also binds to cyclophilin D to block the opening of the mitochondrial permeability transition pore (MPTP), thereby inhibiting epithelial cell apoptosis. With long-term use topical CsA increases tear production and goblet cell density. Commercially distributed as Restasis, CsA 0.05% is packaged without preservatives in single-use vials and twice daily dosing (Hessen and Akpek 2014).

Clinically, pulsed use of corticosteroid drops are often used off label for DED as they have been shown to improve the efficacy of artificial tears or punctal plugs alone. Corticosteroids have multiple mechanisms of action. They increase synthesis of lipocortin A, which suppresses phospholipase A2, an early step in the inflammatory cascade. Prostaglandin synthesis is halted at the levels of phospholipase A2 and cyclooxygenase (COX-1 and COX-2), thereby inhibiting local leukocyte adhesion and chemotaxis, as well as systemic inflammatory responses such as vasodilation and vascular permeability. Steroids also inhibit nuclear factor-kB (NF-kb), a transcription factor that promotes synthesis of proinflammatory molecules, thereby stimulating lymphocyte apoptosis; likewise, they decrease the production of inflammatory mediators on a genomic level (Hessen and Akpek 2014). In murine models topical steroids protect the integrity of corneal epithelial tight junctions, prevent desquamation of epithelial cells, and decrease MMP-9 levels, thereby preserving barrier function (International Dry Eye Workshop (DEWS) Management and Therapy 2007). In humans, pulsed dosing of loteprednol 0.5% (an ester steroid) starting with use 4 times daily for 1 week, followed by a slow taper, has been shown to improve bulbar conjunctival hyperemia and central corneal fluorescein staining scores by over 25% (Pflugfelder et al. 2004). Loteprednol and fluorometholone, a ketone steroid, have also been found to convey lower risk of elevated intraocular pressure when compared to other ketone steroid drops, prednisolone and dexamethasone.

In the case of artificial tears, cyclosporine and steroid drops, innate and active immune protection against microbial invasion may be compromised. For example, the antimicrobial peptides of the innate immune system lose their ability to kill Pseudomonas aeruginosa in the presence of carboxymethyl cellulose solutions in vitro. Cyclosporine has been found, in vitro, to inhibit the production of cytokines involved in wound healing, and increase susceptibility of epithelial cells to viral infection by reducing interleukin production; in human corneal epithelial cells, it has been shown to inhibit cell proliferation (Narayanan et al. 2013). Despite the potential drawbacks these agents remain the mainstay of treating DED.

In cases of meibomian gland dysfunction, the goal of all treatments is to improve the flow of meibom secretions, and a stepwise approach to treatment is employed to minimize antibiotic exposure. Warm compresses are used to dilate meibomian gland orifices, lid scrubs exfoliate debris from the lid margin, and lid massages coax secretions from inspissated glands, all in order to clear the pathway for oil flow. Washing the lid margin with dilute soap also decreases bacterial colonization, which has been shown to inhibit conjunctival goblet cell proliferation and increase the breakdown of meibomian lipid (International Dry Eye Workshop (DEWS) Management and Therapy 2007; Gilbard 2005). While these are valuable and

proven tools for mild to moderate DED, more severe disease that is resistant to treatment may merit antibiotic therapy. Oral tetracyclines are commonly used to treat DED given their dual impact of broad spectrum antibacterial prophylaxis (though only minocycline and doxycycline are able to reach an effective concentration on the ocular surface), and anti-inflammatory effect, achieved through reduction of MMPs, IL-1α and TNF-α (Narayanan et al. 2013). A new approach to treatment of MGD is LipiFlow, in-office thermodynamic treatment for obstructed meibomian glands. The device is a disposable apparatus that is inserted under a patient's upper and lower eyelids; it transfers heat to the palpebral conjunctiva while applying graded, pulsatile pressure to the outer eyelid to express meibom from glands (Finis et al. 2014; Lane et al. 2012).

For both ADDE and EDE, non-pharmacologic tear preservation can be achieved through a longer-acting approach, such as punctual occlusion, or a physical barrier, such as moisture chamber spectacles and contact lenses. Punctal plugs act by increasing longevity of tears in the conjunctival sac. Plugs are dumbbell shaped, with a wide collar that rests at the puncta, a narrow segment that extends into the canaliculus, and a bulb at the end to anchor the plug internally. Temporary plugs are absorbable, made of collagen and lasting a couple months; these are often used to determine if a more permanent plug would be an effective treatment. In contrast, semi-permanent plugs are made of silicone or polymers. The complications of the procedure are few: excessive tearing (usually only if the upper and lower punctum are both occluded), development of a pyogenic granuloma, canaliculitis, or dacryocystitis. Tear retention is also the goal of moisture chamber spectacles, or contact lenses (such as silicone rubber lenses and rigid gas permeable scleral lenses) (International Dry Eye Workshop (DEWS) Management and Therapy 2007).

9 Allergic Conjunctivitis

As atopic diseases became more prevalent in the latter half of the twentieth century, so has allergic conjunctivitis (AC), which is estimated to impact up to 40% of the US population (Singh et al. 2010). AC encompasses several distinct conditions: seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), and giant papillary conjunctivitis.

Diagnosis of DES requires differentiation from other common ocular surface inflammatory conditions. Because patients often find it difficult to characterize their discomfort, a nuanced history of symptoms can help elucidate whether the quality of pain is "burning" (more typical of DES) or "itching" (more specific for AC).

In SAC and PAC, allergens interact with IgE bound to sensitized mast cells, causing cross-linking of IgE at the mast cell membrane with subsequent degranulation and release of histamine, tryptase, prostaglandins, and leukotrienes. This early phase reaction lasts 20–30 min.

Mast cell degranulation also induces a late phase reaction by activating vascular endothelial cells that promote expression of chemokine and adhesion molecules,

including monocytes chemotactic protein-1 (MCP-1), intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM), p-Selectin, and chemotactic factors IL-8 and eotaxin. These mediators recruit activated inflammatory cells (eosinophils, neutrophils, and T lymphocytes) to the conjunctiva. This late phase reaction is prolonged and plays a role in more severe forms of AC, with clinical manifestations of conjunctival injection, itching, chemosis, and conjunctival papillae found on exam (Bonini et al. 2009).

Because the conjunctiva has direct exposure to the environment and an abundant vascular supply to deliver immune mediators, SAC and PAC are common conditions that affect people of all ages. Seasonal allergies account for nearly 90% of all AC, and the most common allergens are airborne pollens that reach peak concentration during the spring and summer. Perennial exposures such as dust mites and mold can also stimulate the same ocular surface response.

AKC and VKC, in contrast, do not involve sensitization of immune mediators to specific environmental exposures and often involve the cornea as well (keratoconjunctivitis). TH2 lymphocytes are thought to play a role in the pathophysiology of these conditions by producing inflammatory cytokines IL-4 and IL-13, which are found in abundance in patients with AKC and VKC; this is a common pathway among allergic disorders.

VKC is a chronic inflammatory condition, most commonly affecting males (2:1 ratio) in tropical climates, that is characterized by broad "cobblestone" papillae on the upper tarsal conjunctival surface, mucus discharge, severe itching, and photophobia. Eosinophilic infiltration of the cornea may lead to the development of a well-circumscribed sterile epithelial "shield ulcer" with underlying stromal opacification, that can cause scarring even after resolution. "Tranta's dots" are collections of necrotic eosinophils, neutrophils, and epithelial cells that are found in crypts along the limbus in active disease. Epithelial cells may also release toxic mediators that compound this injury with macroerosions and plaques.

An estimated 15–20% of the US population has atopy, a genetic predisposition to developing a heightened immune response to common allergens. AKC is the ocular corollary of the atopic conditions of asthma and eczema, which are present in 95% and 87% of patients with AKC, respectively (Guglielmetti et al. 2010). A family history of atopy is often positive. The pathophysiology involves IgE-mediated chronic mast cell degranulation and TH1 and TH2 lymphocyte derived cytokines, which cause severe itching, chemosis, and redness, leading to conjunctival scarring and atopic cataracts if uncontrolled. Like VKC, AKC patients may also have giant papillae and Tranta's dots, but AKC more commonly affects patients in their late teens through the 5th decade of life, while VKC usually resolves by age 20.

9.1 Diagnosis

Allergic conjunctivitis is a clinical diagnosis. Referral to an allergist is essential for systemic workup, including allergen skin testing (scratch test or intradermal injections) and in-vitro IgE antibody tests.

9.2 Treatment

Primary treatment of PAC involves allergen identification and avoidance. Cold compresses may provide symptomatic relief. The primary topical therapy for all types of AC is artificial tears, which mechanically protect and flush the ocular surface of immune mediators.

Traditional topical pharmacologic therapy for allergic conjunctivitis includes mast cell stabilizers, H-1 receptor antagonists, and combination mast cell stabilizers with H-1 receptor antagonists, nonsteroidal anti-inflammatories (NSAIDs), and steroids. Mast cell stabilizers prevent degranulation (via an unclear mechanism) but require a loading dose before reaching effective concentration and therefore have a delayed effect. The pharmacology of antihistamines is correspondingly insufficient in resolving symptoms when used as monotherapy. Because these agents reversibly block only H-1 receptors, leaving other inflammatory mediators uninhibited, they provide rapid but only temporary symptomatic relief.

In the past decade multimodal agents have become the mainstay of therapy because they couple the effect of H1 antagonists and mast cell stabilizers with other anti-inflammatory mechanisms. For example, a broader anti-inflammatory effect is achieved with Azelastine, a selective second generation H1 receptor antagonist that blocks intercellular adhesion molecules (ICAMs), and Epinastine, which blocks H2 receptors and thereby reduces eyelid swelling.

If symptoms persist despite first line treatment supplemental agents may be added. NSAIDs inhibit cyclooxygenase, reducing conjunctival redness and itching mediated by prostaglandins D2 and E2. Corticosteroid drops more directly treat AC by antagonizing NFκB, TGF-β, and activating T-lymphocytes into TH2.

Severe cases require systemic therapy, such as oral antihistamines and less commonly intranasal corticosteroids, in complement to local treatment. Allergen-specific immunotherapy by subcutaneous injection (or sublingual administration) may benefit patients with detectable IgE antibodies to known allergens.

10 Bacterial Conjunctivitis

Conjunctivitis is a nonspecific term that describes inflammation of the conjunctiva which may be caused by a wide range of conditions. AC is the most common etiology, but bacterial, viral, chemical, and toxic conjunctivitis also occur frequently and present with overlapping symptoms. Viral conjunctivitis occurs more frequently in the summer and is the most common cause of infectious conjunctivitis overall and among adults. Bacterial conjunctivitis (BC), which occurs more frequently in the winter, is the most common form among children (50–75% of cases) and the second most common cause in adults (Høvding 2008).

The nonspecific symptoms of chronic BC allow it to masquerade as DES or AC. In a cohort study of 184 culture-positive cases, 58% of patients reported itching, 65% burning, and 35% serous or no discharge. Three signs were found to be strongly predictive of bacterial etiology: bilateral mattering of the eyelids, lack of

itching, and no prior history of conjunctivitis. Notably the type of discharge did not correlate with etiology (Rietveld et al. 2004).

BC may be secondary to a systemic condition, such as in gonorrhea, chlamydia, graft-versus-host disease, and Reiter syndrome. Local BC may be transmitted oculogenitally, contaminated fingers or fomites.

BC may be subdivided into hyperacute, acute, and chronic forms depending on severity and speed of onset. Hyperacute BC is distinguished by rapid onset, profuse purulent discharge, lid swelling, and decreased vision. It is most commonly caused by Neisseria gonorrhoeae, which is treated with intramuscular ceftriaxone. Testing for coexisting genital chlamydial infection is requisite as it is positive in 54% of men and 74% of women (Azari and Barney 2013).

In contrast, acute BC lasts a little over a week and is characterized by conjunctival injection, mucopurulent discharge, and ocular discomfort. In adults the most common isolated pathogens are Staph aureus, Streptococcus pneumoniae, and Haemophilus influenzae, while in children the most common culprits are H. influenzae, S. pneumoniae, and Moraxella catarrhalis.

Chronic BC presents similarly but lasts more than 4 weeks. Its most common pathogens are S. aureus, Moraxella lacunata, and enterics (Azari and Barney 2013).

10.1 Diagnosis

Practitioners should maintain a high degree of suspicion when contact lens wearers present with symptoms of conjunctivitis because this population is more likely to develop conjunctivitis caused by gram negative pathogens. Slit lamp exam should include a thorough evaluation of corneal integrity and the tarsal conjunctiva. Papillary and membranous conjunctivitis suggest a bacterial cause. If there is mucus discharge, it should be cultured to test for both viral and bacterial growth.

10.2 Treatment

A Cochrane meta-analysis that reviewed the treatment of suspected acute BC in 3,673 patients from 11 randomized clinical trials demonstrated that topical antibiotics improve the 5-day remission rate by only 31% compared with placebo. Many cases are self-limited, as clinical remission occurred by days 2–5 in 64% of those treated with placebo. Treatment with antibiotics was, however, associated with significantly better rates of clinical remission by days 2–5 (RR = 1.31), with possible benefit for late clinical remission (by days 6–10 RR = 1.27, with 95% CI = 1.00–1.61). These data suggest a high degree of overtreatment of acute infectious conjunctivitis with antibiotics. Notably, there were no serious adverse sight-threatening outcomes in any placebo group (Sheikh and Hurwitz 2001).

Clinically, topical antibiotics are indicated in patients with contact lens history, ocular surface diseases, corneal trauma, use of immunosuppressive medications, or history of ocular surgery. One large systematic review of 40 studies found that topical antibiotics had higher rates of clinical and microbiological remission in patients with positive bacterial culture, while only microbiological remission was significantly improved in patients with clinically suspected BC (Epling 2010). Patients with culture-positive results should be treated similarly, as well as those with suspicion for more aggressive pathogens that can penetrate an intact cornea (e.g., N gonorrhoeae, H influenzae, Corynebacterium diptheriae, and Listeria monocytogenes).

For adult patients with suspected but uncultured BC, who do not fall into a high risk group or have evidence of ulcerative keratitis, empiric treatment with broad spectrum topical antibiotics may be beneficial. Because the benefit of topical antibiotics is short lived and decreases the duration of symptoms without altering the outcome, some practitioners recommend antibiotics only if symptoms persist beyond 1–2 days. When conjunctivits is suspected to be bacterial but does not respond to appropriate therapy, chlamydial conjunctivitis should be tested for and empirically treated with a single dose of azithromycin, given its varied but often smoldering presentation and potential for scarring.

References

Azari AA, Barney NP (2013) Conjunctivitis: a systematic review of diagnosis and treatment. JAMA 310(16):1721–1729

Bonini S, Sgrulletta R, Coassin M, Bonini S (2009) Allergic conjunctivitis: update on its pathophysiology and perspectives for future treatment. In: Pawankar R et al (eds) Allergy frontiers: clinical manifestations. Springer

Dartt D, Willcox M (2013) Complexity of the tear film: importance in homeostasis and dysfunction during disease. Exp Eye Res 117:1–3

Epling J (2010) Bacterial conjunctivitis. BMJ Clin Evid 2010:0704

Fernandez CA, Galor A, Arheart KL, Musselman DL, Venincasa VD (2013) Dry eye syndrome, posttraumatic stress disorder, and depression in an older male veteran population. Invest Ophthalmol Vis Sci 54(5):3666–3672

Finis D, Hayajneh J, König C, Borrelli M, Schrader S, Geerling G (2014) Evaluation of an automated thermodynamic treatment (LipiFlow®) system for meibomian gland dysfunction: a prospective, randomized, observer-masked trial. Ocul Surf 12(2):146–154

Galor A, Feuer W, Lee DJ, Florez H, Faler AL et al (2012) Depression, post-traumatic stress disorder, and dry eye syndrome: a study utilizing the national United States Veterans affairs administrative database. Am J Ophthalmol 154(2):340–346

Gayton JL (2009) Etiology, prevalence, and treatment of dry eye disease. Clin Ophthalmol (Auckland, NZ) 3:405–412

Gilbard JP (2005) The diagnosis and management of dry eyes. Otolaryngol Clin North Am 38: 871–885

Guglielmetti S, Dart JKG, Calder V (2010) Atopic keratoconjunctivitis and atopic dermatitis. Curr Opin Allergy Clin Immunol 10:478–485

Hallak JA, Jassim S, Khanolkar V, Jain S (2013) Symptom Burden of patients with dry eye disease: a four domain analysis. Shukla D (ed) PLoS ONE 8(12):e82805

Hessen M, Akpek EK (2014) Dry eye: an inflammatory ocular disease. J Ophthalmic Vis Res 9(2): 240–250
Hosaka E, Kawamorita T, Ogasawara Y et al (2011) Interferometry in the evaluation of precorneal tear film thickness in dry eye. Am J Ophthalmol 151(1):18–23
Høvding G (2008) Acute bacterial conjunctivitis. Acta Ophthalmol 86(1):5–17
International Dry Eye Workshop (DEWS) (2007) Diagnostic methodology: methodologies to diagnose and monitor dry eye disease. Report of the diagnostic methodology Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 5:108–152
International Dry Eye Workshop (DEWS) Definition and Classification (2007) The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international dry eye workshop. Ocul Surf 5:75–92
International Dry Eye Workshop (DEWS) Epidemiology (2007) The epidemiology of dry eye disease: report of the epidemiology subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 5:93–107
International Dry Eye Workshop (DEWS) Management and Therapy (2007) Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 5:163–178
Johnson ME (2009) The association between symptoms of discomfort and signs in dry eye. Ocul Surf 7(4):199–211
King-Smith PE, Fink BA, Fogt N, Nichols KK, Hill RM, Wilson GS (2000) The thickness of the human precorneal tear film: evidence from reflection spectra. Invest Ophthalmol Vis Sci 41(11):3348–3359
Lane SS, DuBiner HB, Epstein RJ et al (2012) A new system, the LipiFlow, for the treatment of meibomian gland dysfunction. Cornea 31(4):396–404
Lemp MA (1995) Report of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eye. CLAO J 21:221–232
Li M, Gong L, Sun X, Chapin WJ (2011) Anxiety and depression in patients with dry eye syndrome. Curr Eye Res 36(1):1–7.(4, 5, 17)
Mertzanis P, Abetz L, Rajagopalan K, Espindle D, Chalmers R et al (2005) The relative burden of dry eye in patients' lives: comparisons to a U.S. normative sample. Invest Ophthalmol Vis Sci 46:46–50
Narayanan S, Redfern RL et al (2013) Dry eye disease and microbial keratitis: is there a connection? Ocul Surf 11(2):75–92
Nichols KK, Nichols JJ, Mitchell GL (2004) The lack of association between signs and symptoms in patients with dry eye disease. Cornea 23(8):762–770
Peters E, Colby K (2013) Chapter 3: the tear film. Duane's ophthalmology, foundations, vol 2. Lippincott Williams & Wilkins, Philadelphia. Acccessed online Dec 2016. URL: http://www.oculist.net/downaton502/prof/ebook/duanes/pages/v8/v8c003.html
Pflugfelder SC, Maskin SL, Anderson B et al (2004) A randomized, double-masked, placebo-controlled, multicenter comparison of loteprednol etabonate ophthalmic suspension, 0.5%, and placebo for treatment of keratoconjunctivitis sicca in patients with delayed tear clearance. Am J Ophthalmol 138(3):444–457
Rietveld RP, ter Riet G, Bindels PJ, Sloos JH, van Weert HC (2004) Predicting bacterial cause in infectious conjunctivitis. BMJ 329(7459):206–210
Schaumberg DA, Sullivan DA, Buring JE, Dana MR (2003) Prevalence of dry eye syndrome among US women. Am J Ophthalmol 136(2):318–326
Sheikh A, Hurwitz B (2001) Topical antibiotics for acute bacterial conjunctivitis: a systematic review. Br J Gen Pract 51:473–477
Singh K, Axelrod S, Bielory L (2010) The epidemiology of ocular and nasal allergy in the United States, 1988–1994. J Allergy Clin Immunol 126(4):778–783
Stevenson W, Chauhan SK, Dana R (2012) Dry eye disease: an immune-mediated ocular surface disorder. Arch Ophthalmol 130(1):90–100

Sullivan BD, Whitmer D, Nichols KK, Tomlinson A, Foulks GN, Geerling G, Pepose JS, Kosheleff V, Porreco A, Lemp MA (2010) An objective approach to dry eye disease severity. Invest Ophthalmol Vis Sci 51(12):6125–6130

Tibrewal S, Ivanir Y, Sarkar J, Nayeb-Hashemi N, Bouchard CS, Kim E, Jain S (2014) Hyperosmolar stress induces neutrophil extracellular trap formation: implications for dry eye disease. Invest Ophthalmol Vis Sci 55(12):7961–7969

Tsubota K, Yamada M (1992) Tear evaporation from the ocular surface. Invest Ophthalmol Vis Sci 33:2942–2950

Weisenthal RW, Afshari NA, Bouchard CS, Colby KA, Rootman DS, Tu EY, de Freitas D (2015) 2015–2016 basic and clinical science course section 8: external disease and cornea. American Academy of Ophthalmology, San Francisco, p 45

Whitcher JP, Shiboski CH, Shiboski SC et al (2010) A simplified quantitative method for assessing Keratoconjunctivitis Sicca from the Sjögren's syndrome international registry. Am J Ophthalmol 149(3):405–415

Anti-Infective and Anti-Inflammatory Pharmacotherapies

Mark I. Rosenblatt

Contents

M.I. Rosenblatt (✉)
Department of Ophthalmology and Visual Sciences, Illinois Eye and Ear Infirmary, University of Illinois at Chicago, Chicago, IL, USA
e-mail: mark.i.rosenblatt@gmail.com

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2017_26

Abstract

Ocular infection and inflammation are common and are associated with myriad ocular conditions ranging from mild disease to blinding conditions. There are numerous anti-invectives with spectra against inciting pathogens. Given the potential for ocular infections to rapidly progress initial broad spectrum therapy is usually required, with therapy tailored as microbiological identification and sensitivities become available. The emergence of antibiotic resistance has become a major health problem. Anti-inflammatory therapy can be institutes to prevent ocular symptoms and end-organ damage. A therapy should be selected which is potent enough to interrupt the inflammatory cascades in play, but which avoids potential side effects. Glucocorticoids, NSAIDs, and biological agents may be used singularly or in combination. New drug delivery devices may allow for better local treatment of chronic ocular inflammation.

Keywords

Antibiotic • Anti-infective • Anti-inflammatory • Anti-viral • Glucocorticoids • NSAIDS

1 Introduction

Anti-infective pharmacological therapies are amongst the most common therapies delivered for ocular conditions. The eye is susceptible to a number of ocular infections from various etiologies including bacteria, viruses, and fungi. The use of anti-infective agents in a setting of acute ocular infections can be sight-saving. In addition to the use of anti-infectives in the setting of active infection, the eyes innate defense mechanisms are perturbed during ocular surgeries, where bacteria and other agents may be allowed to enter the eye. Therefore, a very common use of anti-infective therapy is prophylaxis perioperatively (Grzybowski et al. 2016).

While this chapter will discuss, at least in brief, a number of anti-infective therapies, the chapter will also serve to highlight some of the principles behind the use of anti-infective therapies both in the treatment of active infection and prophylaxis.

2 Anti-Infective Drug Delivery

In addition to the systemic use of antibiotics, as one be used for a number of systemic infections, the eye lends itself to other modes of anti-infective administration, including topical, sub-conjunctival, intracameral, intravitreal, and peri-ocular.

The majority of anti-infective therapy is indeed delivered in the form of eye drops. As discussed elsewhere in this textbook, the pharmacodynamics of ocular drug therapy can be quite complex. With respect to the use of anti-infectives, the ability for antibiotic concentrations to reach the minimum inhibitory concentration

(MIC) for a suitable amount of time to allow for bacteria killing is necessary. Thus, the initial concentration of an anti-infective in an eye drop, the clearance and dilution of this anti-infective, and the ability of the anti-infective to penetrate the cornea in drop form will all be important factors in determining the effectiveness of a particular topical therapy. Indeed, the initial concentrations delivered in eye drop form exceed by many fold the concentrations that would be achievable in serum through either oral or parenteral administration. Of course, the overall ability of the high concentration of delivered anti-infective to reach the target tissue will be highly dependent upon the specific nature of the pharmacological agent, with factors such as hydrophilicity, and hydrophobicity being extremely important.

The use of eye drops is in some ways the most convenient form of antibiotic administration, given for most patients that relative ease of eye drop instillation. In cases of severe infection, administration every 30 min to 1 h may be required (as in the case of treatment of a bacterial corneal ulcer) and this convenience would be abrogated. Indeed, compliance, even in the setting of severe infection for antibiotic drops administered at such frequencies diminishes considerably. On the other hand, in the case of peri-operative use of antibiotics following common procedures such as cataract surgery, the use of antibiotic drops at a QID frequency is relatively well-tolerated by patients, especially given that they are administering other anti-inflammatory drops at similar frequencies.

The use of anti-infective therapies delivered subconjunctivally is not as common, but may be used in select cases. For patients who may have difficulty administering eye drops, or patients at the end of a surgical procedure who may be patched for several hours, the administration of subconjunctival anti-bacterial agents is not uncommon. By use of injection under the conjunctiva, a reliable initial dose is administered, however the pharmacodynamics in the medium to longer term can be quite variable and dependent upon a precise site of injection and other patient factors.

Intravitreal anti-infective therapy is used in the setting of acute intraocular infection, particularly when very high levels of antibiotic are required to clear infections in the vitreous. Indeed, for endophthalmitis the use of intravitreal antibiotics is the preferred method of drug delivery. These antibiotics can be injected at the same time as a sample tap is taken for microbiological evaluation. The application of intracameral at the end of a surgical procedure is also possible, and a large multi-site trial in Europe has shown that the use of cefuroxime eye drops at the end of surgery is potentially capable of reducing post-operative endophthalmitis in routine cataract surgery.

3 Anti-Infective Drug Prophylaxis

The use of anti-infective therapy, as mentioned above, is quite common for prophylaxis of infection following intraocular surgeries. Intraocular surgery is one of the most common surgeries performed, but despite the common performance of these procedures relatively scant data is available regarding many of the

measures used to prevent infections after surgery. Meta-analysis regarding measures that can prevent endophthalmitis following cataract surgery (the most commonly performed intraocular procedure) has identified the scrubbing of the eyes with a povidone iodine solution as one effective therapy, and more recent data from a European consortium suggests that intracameral application of cefuroxime can also prevent bacterial endophthalmitis. A major barrier in evaluating the effectiveness of preventative measures against endophthalmitis is that the rates of endophthalmitis are overall quite low. Thus large populations must be studied in a rigorous way, and the number needed to treat to have efficacy is quite often very large. Currently, fluoroquinolones are the most commonly used prophylactic antibiotic for use in cataract surgery (they are either applied only postoperatively or for a few days preoperatively as well as postoperatively). It should be noted that the FDA indications for use of fluoroquinolone antibiotics are not for prophylaxis after cataract surgery, but are instead primarily for the treatment of bacterial conjunctivitis. This example of off-label use of antibiotics is quite common, and given that the use of these prophylactic antibiotics are standard of care in many communities, their use in these cases is likely substantiated.

4 Selection of Anti-Infective Drug Therapy

Ocular infections by a number of infectious agents, and perhaps most especially bacteria can be devastating. Once the eye's protections have been broken down by an ocular infection, bacteria can rapidly cause damage to ocular tissues through mechanisms directly related to bacteria (including release of proteases) and secondary effects of inflammation related to infection are possible. Indeed, more virulent organisms causing corneal ulceration or bacterial endophthalmitis can cause either corneal perforation or substantial retinal damage in a matter of just a few hours. Therefore, initial antibiotic therapies are often instituted without positive culture results or positive staining in a microbiology laboratory. In the absence of this defined data, broad antibiotic coverage is often instituted for ocular disease, and generally includes at least one agent with potent gram-positive coverage as well as an agent with potent gram-negative coverage. This would include the use of fortified antibiotics used with high-frequency in the case of corneal ulceration, as well as the injection of multiple antibiotics in cases of suspected endophthalmitis. For less severe, and perhaps more indolent infections of the anterior segment single agents may be the initial choice. The use of commercially available single agents, such as fluoroquinolones in the initial treatment of bacterial keratitis when ulceration is small and not in the central visual access may be appropriate. Without adequate response to these initial single agent therapies, however, more broad treatment should be instituted. As additional culture and staining information becomes available the anti-infective therapies can be tailored to more adequately treat the agent responsible, including using multiple agents with varied mechanisms of action to treat the microbial culprits. The possibility of poly microbial infection should continue to be considered and may warrant continued use of broad-spectrum

antibiotics even after initial identification of a single, perhaps more dominant organism (Lakhundi et al. 2017).

Many anti-infectives are able to provide robust killing or static effects on pathogens. Targeted pathogens may ultimately acquire resistance. Indeed, the problem of resistance to a variety of anti-infective agents is increasing, and in many cases has become a public health problem. While the promiscuous use of antibiotics in general has been a leading force in generating this resistance, there is not significant evidence that resistant strains result commonly from specific ocular treatment. It seems that the bulk of resistant infections found within the eye come from bacteria that have become resistant from non-ocular tissues and non-ocular use of primarily systemic anti-infectives.

Resistance of bacteria to commonly prescribed antibiotics has received the most attention. A significant number of strains are now methicillin resistant (MRSA). While this antibiotic resistance of Staph aureus was in the past primarily confined to the hospital setting, there are now substantial amounts of MRSA found within the community. This increasing antibiotic resistance of *Staphylococcus aureus* has led to alternative guidelines in many communities to begin treating suspected or proven Staphylococcal infections with vancomycin as first line therapy pending availability of isolate susceptibility data.

The resistance to fluoroquinolone antibiotics is also increasing in frequency. Substantial numbers of community and in-hospital isolates of gram-negative bacteria are now being found to have resistance to fluoroquinolones. This fluoroquinolone resistance is perhaps even more troubling for ophthalmologists, as fluoroquinolones are often the first-line agents in terms of both therapy and prophylaxis as described above.

The emergence of increasing numbers of resistant strains of bacteria further emphasizes the need to obtain appropriate cultures when encountering frank ocular infections. While in the past the presumptive use of a fluoroquinolone or cephalosporins in a community acquired condition may have seemed appropriate, the opportunity to obtain specimens and to learn of antibiotic resistance/susceptibility early in the course of the disease may now be significantly more important.

5 Antiviral Resistance

Resistance to antiviral agents is also possible. Viruses, including the herpes simplex virus, may mutate with some frequency, and the potential targets for commonly used agents, such as acyclovir, used to treat herpes simplex virus (HSV) may generate resistance. The occurrence of resistance of HSV primarily rests in patients who are immunocompromised, including patients with AIDS or following bone marrow transplantation. The occurrence of antiviral resistance in immunocompetent patients is still quite low. The use of alternative antiviral agents with non-thymidine kinase-dependent targets is required in cases of antiviral resistance. New microbiological and molecular techniques for identifying this resistance have

been developed and are in the process of being further improved (Tsatsos et al. 2016).

6 Antifungal Resistance

The significance and prevalence of antifungal resistance is less well understood. Fungal disease often presents in an indolent fashion and can be in general more difficult to treat than either infections from bacterial or viruses. The susceptibility that has been identified has primarily occurred for species of candida, a pathogen which can severely affect the eye.

7 Specific Anti-Bacterial Agents (Table 1)

7.1 Fluoroquinolones

Fluoroquinolones are fluorinated chemical structures derived from nalidixic acid. As mentioned above these are very commonly used anti-infective agents, both for the treatment of active infection and prophylaxis. Fluoroquinolones' mechanism of action is the targeting bacterial DNA synthesis through the inhibition of DNA gyrase and topoisomerase IV. Through their action on these two separate targets they are able to alter bacterial DNA supercoiling and prevent bacterial replication. Fluoroquinolones have been developed as several generations but overall have a broad spectrum with activity against both gram-positive and gram-negative bacteria. The earlier generations of fluoroquinolones were more potent against gram-negative bacteria, however the fourth generation of fluoroquinolones have significant activity against gram-positive organisms and atypical mycobacteria. Currently available fluoroquinolones are available as topical solutions and include levofloxacin, gatifloxacin, moxifloxacin, ciprofloxacin, and ofloxacin. Although approved with FDA indications for the treatment of bacterial conjunctivitis, they also are commonly used in the treatment of corneal ulcers. Most patients having cataract surgery also receive fluoroquinolones to potentially prevent the occurrence of endophthalmitis.

Fluoroquinolones have the advantage of being extremely well-tolerated with excellent rates of penetration into ocular tissues and sustained concentrations above minimum inhibitory concentrations for many bacteria throughout ocular tissues and in the tears. In the setting of penetrating trauma, the use of oral fluoroquinolones is common following repair to prevent trauma-related endophthalmitis. The use of oral as opposed to parenteral antibiotics allows for appropriate and effective control of infection risks without the need for IV drug administration.

Table 1 Classes of anti-bacterial agents

Class	Pharmacotherapy examples	Mechanism of action
Fluoroquinolones	Ciprofloxacin	Inhibits DNA gyrase and topoisomerase IV
	Ofloxacin	
	Gatifloxacin	
B-lactam		Inhibits bacterial cell wall synthesis
Penicillins	Penicillin G	
	Amoxicillin	
	Methicillin	
	Piperacillin	
Cephalosporins		
1st gen	Cefazolin	
	Cephalexin	
2nd gen	Cefuroxime	
	Cefoxitin	
3rd gen	Cefotaxime	
	Ceftriaxone	
4th gen	Cefpirome	
Macrolides	Erythromycin	Inhibits protein synthesis
	Azithromycin	
	Clarithromycin	
Tetracyclines	Doxycycline	Inhibits protein synthesis
	Minocycline	
Other agents		
Vancomycin		Inhibits bacterial cell wall formation
Polymixin B		Dissolves cell wall components
Bacitracin		Inhibits bacterial cell wall formation

7.2 Aminoglycosides

Aminoglycosides are chemical agents composed of amino sugars with glycosidic linkages. The aminoglycosides work by binding to and inhibiting the 30S and 50S ribosomal subunits which in turn interferes with protein synthesis. The aminoglycoside antibiotics are highly effective against gram negative bacteria and the susceptibility of bacteria to aminoglycoside antibiotics is highly dependent upon the rate of transport of these agents into the bacteria. Resistance to aminoglycoside antibiotics is somewhat less common than resistance to other antibiotic classes, although there are a large number of non-susceptible strains, particularly those bacteria that are gram positive. Commonly used aminoglycoside antibiotics include kanamycin, amikacin, tobramycin, and gentamicin.

The aminoglycosides are given either as eye drops or less frequently as periocular injections or as intravenous agents. Aminoglycosides have been used for intravitreal administration as well in the setting of gram negative endophthalmitis. The toxicity

profiles of aminoglycoside antibiotics are less favorable than for the aforementioned fluoroquinolones and toxicity to ocular structures at higher aminoglycoside doses has been noted.

7.3 Beta-Lactams

The beta-lactam containing antibacterial agents include both the penicillins and cephalosporins. The mechanism of actions of both penicillins and cephalosporins is the inhibition of bacterial subwall synthesis through the inactivation of bacterial transpeptidases.

Various penicillins and cephalosporins have been developed over time with earlier available penicillins and earlier generations of cephalosporins having high activity against gram positive organism with the later generations expanding antibacterial coverage and possessing enhanced activity against some gram negative bacteria. The use of penicillin through penicillin G and penicillinase-resistant penicillin such as methicillin and nafcillin is useful in the treatment of susceptible strains of gram positive organisms. Broader spectrum penicillins such as ampicillin have additional coverage and can often be used systemically for soft tissue infections of the eye. Further expansion of penicillin coverage such as that seen with carbenicillin and piperacillin allows for additional activity against pseudomonas and gram negative organisms. Cephalosporin of multiple generations are often also commonly used for ocular disease. Given their high efficacy against gram positive bacteria cephalosporins are frequently used in combination with agents such as tobramycin for the treatment of both corneal ulcers and endophthalmitis at fortified doses.

7.4 Tetracyclines

Tetracyclines including doxycycline and minocycline inhibit the translation of proteins by binding to the 30S subunit of the bacterial ribosome. Doxycycline and minocycline are more active than the tetracycline given their increased lipophilicity. These drugs are bacteriostatic and have broad spectrum against multiple forms of gram positive and gram negative bacteria. These agents are most often given in oral form and have excellent activity against chlamydial infections. Formulation of an eye drop is more difficult as these drugs have poor water solubility. Side effects of oral tetracyclines include a GI upset and light skin photosensitivity.

7.5 Macrolides

Macrolide antibiotics include erythromycin, azithromycin, and clarithromycin. These antibiotics bind to the 50S subunit of bacterial ribosomes and ultimately

interfere with protein translation. Macrolide antibiotics vary in their specificity, with erythromycin having good effect against gram positives and both clarithromycin and azithromycin having expanded coverage against gram negative organisms. These agents are often given orally, although topical formulations of both erythromycin (as an ointment) and azithromycin (as a topical gel) are now available. Topical azithromycin appears to have good penetration into the lid and has been used for its anti-inflammatory effects in the setting of blepharoconjunctivitis.

7.6 Additional Antibiotic and Combinations

A number of combination antibiotic formulations are available. Many of these combinations have a wide spectrum of activity and include agents such as neomycin, polymyxin D sulfate, and sulfacetamide. Polymyxin D sulfate is a set of peptides that function as detergents to dissolve cell membranes. Bacitracin is a peptide mixture which inhibits bacterial cell wall synthesis. Sulfactamide inhibits bacterial synthesis of folic acid.

Vancomycin is a glycopeptide which is bactericidal and has high gram positive specificity. Its mechanism of action is inhibition of polymerization of glycopeptides in the cell wall of gram positive bacteria. The primary use of vancomycin has been in the treatment of staphylococcal infections, especially in patients with either suspected or confirmed methicillin resistant Staph aureus MRSA. Vancomycin is formulated as either a topical solution or as a formulation for intraocular administration. Vancomycin is often a first-line therapy for both severe infectious keratitis and suspected or confirmed endophthalmitis. IV vancomycin may also be used in cases of penetrating ocular trauma, although risk of ototoxicity or nephrotoxicity may be possible. Topical and intraocular vancomycin appears to be well tolerated.

8 Antiviral Drugs

A number of antiviral agents have been developed to help treat serious viral illnesses affecting the eye. While adenoviral conjunctivitis is perhaps the most common viral disease of the eye, currently no effective therapies exist. In contrast, effective therapies for the treatment of herpes simplex and herpes zoster virus have been developed as well as antiviral agents to treat cytomeglovirus (CMV).

Administration of antiviral agents may be either a topical or systemic. Topical administration has the potential advantage of localizing therapy to ocular tissues, while avoiding any non-ocular side effects. High concentrations of topical antiviral drugs may be delivered to the ocular surface. For herpes simplex virus, topical therapy, as supported by the results of the Herpetic Eye Disease Study (HEDS), was the most common form of treatment. Nucleotide analogs which compete for incorporation into viral nucleotides have included idoxuridine and trifluridine. While effective in the treatment of herpetic eye disease, a major disadvantage of

these particular agents has been ocular toxicities including epitheliopathy, ocular surface toxicity, and ocular discomfort. Additionally, these agents require a frequent dosing schedule, up to 9 times per day, to be effective against herpes simplex virus. More recently, alternate therapies using alternate guanine analog, ganciclovir have been developed for topical use. The Ganciclovir gel formulation appears to be better tolerated and requires less frequent administration than the prior forms of topical therapy (Tsatsos et al. 2016).

While the use of systemic therapies for the treatment of corneal epithelial disease was not supported by the HEDS trial, the ease of use of oral antiviral medications as well as a clinical experience in the years following the HEDS trial has made oral antivirals a popular choice for therapy. Oral agents including acyclovir, famciclovir, and valacyclovir have all been used effectively to treat herpetic herpes simplex virus keratopathy. Additionally these agents are effective in the treatment and suppression of herpetic stromal keratitis, a potentially blinding condition. Suppressive doses of these three agents can be used to effectively prevent recurrence of herpes simplex virus in the cornea. The required duration of suppressive therapy using oral antiviral agents has not been firmly established. In patients with multiple recurrences continuous use of these antivirals has been encouraged by many. Acyclovir, famciclovir, and valacyclovir are encouraged by the fact that these drugs are well tolerated in the majority of patients and significant side effects are uncommon. Higher doses of these drugs are required to treat the acute stage of herpes zoster. Notably, valacyclovir is contraindicated in patients who are immunosuppressed given added risk of side effects in this subgroup of patients. Intravenous administration of Acyclovir is indicated for potential cases of neonatal herpes simplex virus infection, as well as for severe cases of herpetic retinitis including cases of acute retinal necrosis.

9 Anti-Fungal Drugs

Ocular infection with fungal disease is a serious and often very difficult to treat condition. Fungal infections of the cornea are usually the result of a direct trauma and inoculation of corneal tissue with fungal elements. A fungal endophthalmitis commonly results from post-surgical or a traumatic insult, but may also result from endogenous spread of fungal elements to the eye, particularly after parenteral nutrition. The difficulty in treating fungal infections is in part due to the fact that fungi are eukaryotes and potential drug targets in these very primitive eukaryotes are often overly similar to similar protein targets in more advanced mammalian eukaryotes, such as the patient.

The currently available fungal agents may be delivered either topically, injected into the diseased tissue, or delivered systemically. Fungal agents often have very poor penetration when delivered topically, so in the absence of a permissive epithelial surface or loss of epithelium the concentrations of antifungal agents within the infected tissues are often not suitably high to kill the offending fungus.

Frequent administration of antifungal drugs topically is often required, particularly in the early stages of therapy (Garg et al. 2016).

Direct injection of antifungal agents may allow for access of drugs to the infected site. Injections can either be done within the corneal stroma, particularly if the epithelium is intact, or injections into the anterior chamber or vitreous cavity when intraocular infection and endophthalmitis are suspected. Anterior chamber antifungal agents are also frequently administered at the time of penetrating keratoplasty for nonresponding fungal keratitis.

Finally many antifungal agents can be given either orally or intravenously. Many of these drugs can achieve reasonably high concentrations, often over the MIC, in ocular tissues. Enhancement of antifungal concentration is frequently seen during infection related inflammation which breaks down some barriers commonly found in non-diseased noninfected ocular tissues.

Methods of action of antifungal agents are primarily through disruption of fungal cell membranes. Polyenes are chemical structures with lipophilic domains which allow them to interact with sterols in the cell membrane of fungus causing membrane damage and fungal cell death. Two commonly used polyenes are amphotericin B and natamycin. Natamycin is currently the only FDA approved antifungal agent and given its low penetration into ocular tissues is often given topically hourly during the early stages of therapy for fungal keratitis. Amphotericin B is formulated for topical use and also requires frequent dosing given its similarly poor entry into corneal tissue. Amphotericin B is effective against a cadre of fungi including aspergillus and candida while natamycin is particularly effective for the treatment of Fusarium infections. Careful microbiological examination of scrapings from infected corneas is required to determine the nature of the fungus and steer the correct course of antifungal agents. The polyenes weakening of the plasma membrane of the fungus can allow for synergistic activity of all the antifungal agents mentioned below.

The imidazole and triazole group of antifungal agents are also damaging to the cell membrane of fungi and appear to disrupt systems within the plasma membrane required for fungal survival. Imidazoles include ketoconazole and miconazole, while the triazols include fluconazole, itraconazole, and voriconazole. Each of these agents seems to have best activities against candida species, but other fungal diseases are also sensitive to subsets of the imidazoles and triazols. Again, isolation of fungal elements with careful microbiological evaluation including sensitivity to these agents is required. Imidazole and triazols are often given by an oral route or through IV and are able to gain good penetration into infected tissues. Finally flucytosine is an antifungal agent that is able to inhibit fungal thymidylate synthase. This mechanism of action is distinct from actions on cell membranes that the other antifungal agents possess. However, the inability of flycytosine to enter many fungal species makes it a less broadly applicable antifungal agent. It appears to have its best activity against both candida and cryptococcus species.

10 Anti-Inflammatory Medications

10.1 Introduction

Ocular inflammation can be incited as a result of myriad pathologies. Acute inflammation in the setting of conditions such as dry eye, chemical injury, or trauma is often symptomatic and may be quite profound. More chronic forms of inflammation may result from uveitis, may be either more indolent or frankly symptomatic. The consequence of activated levels of inflammation in the eye is akin to that seen in inflammation seen classically in other parts of the body. There is increase of vascularization, blood flow, and recruitment of inflammatory cells to the region that is affected. Recruitment of these inflammatory cells can lead to release of inflammatory mediators such as cytokines or arachidonic acid metabolites as well as the release of proteases which can directly destroy tissue. Inflammation is often developed in a cascade like-fashion where initial insults are then amplified by signal transduction mechanisms to accelerate and accentuate the inflammatory conditions. While there exist innate mechanisms to quench inflammatory conditions, often pharmacological intervention is required more immediately to help resolution of the inflammatory condition and to prevent ocular tissue damage. Newer roles for anti-inflammatory medications in controlling angiogenesis and vascular leakage have also been more recently identified, and an anti-inflammatory agent can also be used for conditions such as diabetic macular edema which are not traditionally considered to be inflammatory.

The route of administration of anti-inflammatory agents is often determined by the nature of the underlying inflammatory condition. For superficial inflammation, such as that might be seen in dry eye or allergic conjunctivitis often topical administration of anti-inflammatory agents is suitable. Even in cases of anterior uveitis or posterior disease such as macular edema the use of eye drops, either steroids or NSAIDS can be useful in the long-term given the penetration of these agents.

As with the anti-infectives discussed above, the concentrations of anti-inflammatory agents within eye drops can be well above that generally seen in serum when these agents are administered systemically. Both nonsteroidal anti-inflammatories and glucocorticoids often have outstanding penetration into the eye and concentrations of drugs suitable to treat many types of inflammation can be achieved via the topical route.

For more severe forms of inflammation, or inflammation not adequately treated through topical administration of drops, injections of steroid formulations have proven to be effective therapies. Depot injection of steroid has been an effective therapy for chronic uveitis and retinal edema. A burgeoning area of an anti-inflammatory drug deliver has been the ability to not only inject standard formulations of anti-inflammatory drugs, but to also create sustained release devices capable of eluting drug over several weeks to years. These intravitreally injectable devices have thus far been designed to release potent glucocorticoids

(as will be discussed in greater detail below) but this type of technology should be amenable to delivery of other anti-inflammatory agents.

In highly persistent bouts of inflammation, and especially those bouts of inflammation associated with systemic inflammatory disease the use of systemic anti-inflammatories is often required as treatment. Oral steroids at doses at or above 1 mg/kg are generally well tolerated by patients in the acute setting. Injectable medications may include high-dose intravenous steroids, the injection of very potent immunomodulatory therapy such as cyclophosphamide, or the injection of a biological agents capable of controlling certain specific types of inflammation.

10.2 Combined Use of Anti-Infectives and Anti-Inflammatories in Severe Disease

As mentioned above the consequence of infection can be robust inflammation. The combination of pathogen-derived factors as well as factors emanating from inflammatory cells recruited to the site of infection can lead to significant ocular damage and ultimately a sight-threatening status. The role of potent anti-inflammatories in the setting of concurrent infection has been controversial. Proponents of combining these therapies assert that by preventing a robust inflammatory response and the cascade of inflammatory signaling that can be difficult to control early on in the course of infections using anti-inflammatories, most commonly steroid preparations, that outcomes from infections of the eye can be improved. However, there exists risk that by suppressing the native immune response to infection, clearance of potential pathogens is delayed and that pathogen-based damage is heightened. Over the past several decades the use of combination of anti-infectives and anti-inflammatories has been evaluated in several settings. In the setting of herpetic eye disease, most notably herpes simplex virus infection, there can be both an active replication of virus causing epithelial and stromal disease and a robust stromal keratitis that is immune mediated. The use of corticosteroids without anti-viral therapies may allow uncontrolled, unchecked, or amplification of virus and worsening of disease. However strong evidence exists that the use of topical steroids with a concomitant use of oral anti-virals can allow for quicker resolution of the inflammatory process and the avoidance of potentially blinding, scarring, and neovascularization.

The role of combination in therapy in the management of corneal ulceration has been less definitive. In the Steroids for Corneal Ulcers Trial (SCUT), the use of steroids in acute ulceration was examined (Palioura et al. 2016). This study found an overall lack of benefit, but also a lack of harm in using steroids in conjunction with antibiotics for the treatment of bacterial corneal ulcers. A study of a subset of infections reveals that there may be a potential benefit for using steroids in cases when steroid therapy is initiated very early in the course of the disease for ulcers that are located within the visual axis. The study, however, was not powered appropriately nor designed to rigorously evaluate this hypothesis.

The management of bacterial endophthalmitis is a major challenge, given the very high bacterial load within the vitreous cavity. However, following surgical intervention to clear the vitreous of both bacteria and vitreous gel corticosteroids may be of benefit. The robust inflammation resulting in the vitreous often leads to substantial damage to retinal tissue and the potential setup for proliferative retinopathy and fibrosis later in the resolution of the disease. By combining steroids during the treatment of endophthalmitis there is some evidence that outcomes may be somewhat improved. Detailed selection of which cases are most likely to benefit from this type of intervention has not yet been conclusively identified (Kim et al. 2017).

11 Glucocorticoids

Corticosteroids are a mainstay of ocular anti-inflammatory therapy. Corticosteroids function via binding to glucocorticoid receptors to form complexes which are allowed to bind to the nucleus and alter gene transcription. Thus to be effective glucocorticoids must also be membrane soluble to find their way to their respective receptors. The transcriptional effects of glucosteroid application are myriad and a constellation of genes may be either induced or suppressed to regulate a number of biological processes in cells and tissues. The main role of binding to glucocorticoid receptors is to suppress inflammation including the recruitment and activation of immune cells and regulation of the innate immune system. With application of steroid agents, inflammatory cytokine production as well as arachidonic acid metabolite release (which are mediators of the inflammatory response at the tissue level) is markedly inhibited. Importantly glucocorticoids also suppress the release of enzymes which are able to directly cause tissue damage.

In addition to the anti-inflammatory effects of glucocorticoids these steroid agents may also have additional effects on the eye, many of which are unwanted side effects. Known adverse effects following the application of corticosteroids include ocular hypertension and glaucoma, the formation of cataracts (particularly of the posterior subcapsular type), potential scleral melting owing to the inhibition of collagen synthesis, and exacerbation of concomitant infections due to immunosuppression. More generally, steroids supplied systemically can have additional effects including hyperglycemia and diabetes, gastritis and gastric ulceration, altered mental status, other metabolic abnormalities and obesity, and an idiosyncratic aseptic necrosis of the hip. Thus, in addition to the many useful attributes of this class of drug, the relatively nonspecific nature of steroid action makes it susceptible to complicating events.

There are a number of commercially available glucocorticoid preparations, which can be delivered as topical solutions or suspensions, periocular injections, intraocular injections, or delivered systemically via the oral or intravenous routes. The relative potencies of steroids can vary markedly and is at least partially determined by the affinity of the glucocorticoids for their receptors and the ability of bound receptors to alter the transcription of inflammatory mediators

(Table 2). Clinically, practitioners prefer to choose a steroid that has an adequate potency to achieve control of the inflammatory process, but with the lowest-needed potency so that unwanted side effects are avoided or mitigated. For exacerbations of more common diseases like dry-eye syndrome or allergy, weak topical corticosteroids are very effective agents. For uveitis, often higher doses of topical steroids or periocular steroids are required to control significant processes. For control of significant retinal and posterior segment inflammation intraocular steroid application may be required. And for a disease such as multiple sclerosis systemic high-dose intravenous steroid application is the preferred therapy.

As mentioned above, a significant complication of steroid application to the eye, particularly local application of high-dose corticosteroid formulations, is the elevation of intraocular pressure. The mechanism of steroid-induced ocular hypertension is not yet clearly defined, but it does appear to be dependent upon decrease in aqueous outflow facility. The relative intraocular pressure elevations of the various locally applied steroid formulations does not completely mirror the potency of the steroids, although there is a limited correlation. Given the potentially difficult-to-manage complications of ocular hypertension, several newer steroids including rimexolone and loteprednol have been developed which maintain excellent corticosteroid potency, but which have a relatively lower probability of causing ocular hypertension. Of note, ocular hypertension related to corticosteroid application generally occurs after approximately 2 weeks of corticosteroid therapy, and appearance of elevated pressures prior to 1 week of application is highly unusual.

A major advance in the application of corticosteroids for ocular disease has been the development of intraocular implantable drug eluting devices for maintaining sustained levels of corticosteroids to treat chronic disease. A dexamethasone delivering biodegradable polymer provides 6 months of drug release, while two nondegradable implants to elute fluocinolone acetate for up to 3 years have also been developed. Indications for these devices include the treatment of ocular inflammation as well as diabetic macular edema.

Preservative-free triamcinolone acetonide suitable for intraocular injection was approved by the FDA in 2007. This injection of this particulate steroid injection was approved for the visualization during vitrectomy and treatment of ocular inflammatory conditions otherwise unresponsive to topical corticosteroids.

Table 2 Corticosteroid potency

Pharmacotherapy	Relative anti-inflammatory activity
Difluprednate	60
Fluorometholone	40
Dexamethasone	25
Loteprednol	25
Prednisolone acetate	4
Hydrocortisone	1

11.1 Nonsteroidal Anti-Inflammatory Drugs (NSAIDS)

While cortical steroids broadly inhibit inflammation, a number of nonsteroidal anti-inflammatory drugs are available within more specific mechanism of action. A group of NSAIDS are potent inhibitors of inflammation. Through the inhibition of the cyclooxygenase, the NSAIDs are able to prevent the conversion of arachidonic acid into endoperoxides and hydroperoxides, which ultimately can form prostaglandins. The cyclooxygenases are also responsible for the production of thromboxane and prostacyclin which are additional mediators of inflammation. Finally the production of leukotrienes from these intermediates also contributes to profound inflammation, including anaphylaxis (Wilson et al. 2015).

NSAIDs are generally given as topical agents for localized ocular disease, as well as for a low-grade inflammation related to a surgery or mild uveitis. Topical NSAIDs are generally well tolerated with the primary symptoms being an initial discomfort with application, and the potential inhibition of wound healing due to toxicity to the epithelium. Corneal melts related to the toxicity have been reported with the over application of NSAIDS, usually with the existing ocular surface disease. The application of topical NSAIDs is indicated for immediate post-operative inflammation and pain in the setting of cataract surgery, the maintenance of the mydriasis during cataract surgery, and the management of cystoid macular edema particularly following cataract surgery. Perioperative NSAIDs are frequently given, with the use of these agents for up to a month after surgery in uncomplicated cases (Kim et al. 2015). Topical NSAIDs are used in a more limited basis following corneal abrasion or photorefractive keratectomy, however care must be taken not to experience the untoward side effects of delayed wound healing and corneal toxicity.

Oral NSAIDs may be useful in the treatment of systemic disease, or localized ocular disease that is more profound. Specifically, systemic NSAIDs have been used in the treatment of scleritis as well in moderate uveitis. Use of oral nonsteroidal anti-inflammatory also inhibits platelet activity with the potential for increased bleeding with injury.

11.2 Other Systemic Anti-Inflammatory Medications

For the most profound types of inflammation, additional systemic immunosuppressive therapy may be required. While corticosteroids are in many cases able to control the acute phases of even these more severe processes, the aforementioned complications of high dose corticosteroid therapy limit their long-term use in many patients. Immunomodulatory therapy (IMT) using agents such as methotrexate, cyclosporine, cyclophosphamide, is a viable treatment for chronic uveitis. Many of these uveitis syndromes are associated with systemic manifestations including diseases such as rheumatoid arthritis and lupus.

More recently, the class of biologic agents has been used to treat ocular inflammatory disease. New guidelines from the American Uveitis Society recognize the

use of TNF-alpha inhibitors including infliximab, adalimumab, or etanercept for use in treating multiple uveitis syndromes including Behcet's disease, JIA, and anterior uveitis in patients with ankylosing spondylitis. There are numerous biologic agents under development for use in other rheumatologic diseases, and their use for ocular inflammation is likely to increase in the future.

12 Summary

There are myriad pharmacological agents that can be used as either anti-infective or anti-inflammatory agents. The careful selection of these agents to maximize a potential benefit while minimizing untoward side effects is desirable. The increasing amount of incidents of resistance to traditional antibiotic agents makes the judicious use of antibiotics important, but also requires development of additional agents with a broader spectrum of activity and novel mechanisms of action. Traditional use of anti-inflammatories such as glucocorticoids and nonsteroidal anti-inflammatories may be highly effective in treating ocular disease, however steroid use may be limited by significant side effects. The development of immunomodulatory therapies, particularly a new class of biological agents holds great promise for improving the specificity of anti-inflammatory therapy.

References

Garg P, Roy A, Roy S (2016) Update on fungal keratitis. Curr Opin Ophthalmol 27(4):333–339. doi:10.1097/ICU.0000000000000272

Grzybowski A, Kuklo P, Pieczynski J, Beiko G (2016) A review of preoperative manoeuvres for prophylaxis of endophthalmitis in intraocular surgery: topical application of antibiotics, disinfectants, or both? Curr Opin Ophthalmol 27(1):9–23. doi:10.1097/ICU.0000000000000216

Kim SJ, Schoenberger SD, Thorne JE, Ehlers JP, Yeh S, Bakri SJ (2015) Topical nonsteroidal anti-inflammatory drugs and cataract surgery: a report by the American Academy of Ophthalmology. Ophthalmology 122(11):2159–2168. doi:10.1016/j.ophtha.2015.05.014

Kim CH, Chen MF, Coleman AL (2017) Adjunctive steroid therapy versus antibiotics alone for acute endophthalmitis after intraocular procedure. Cochrane Database Syst Rev 2:CD012131. doi:10.1002/14651858.CD012131.pub2

Lakhundi S, Siddiqui R, Khan NA (2017) Pathogenesis of microbial keratitis. Microb Pathog 104:97–109. doi:10.1016/j.micpath.2016.12.013

Palioura S, Henry CR, Amescua G, Alfonso EC (2016) Role of steroids in the treatment of bacterial keratitis. Clin Ophthalmol 10:179–186. doi:10.2147/OPTH.S80411

Tsatsos M, MacGregor C, Athanasiadis I, Moschos MM, Hossain P, Anderson D (2016) Herpes simplex virus keratitis: an update of the pathogenesis and current treatment with oral and topical antiviral agents. Clin Exp Ophthalmol 44(9):824–837. doi:10.1111/ceo.12785

Wilson DJ, Schutte SM, Abel SR (2015) Comparing the efficacy of ophthalmic NSAIDs in common indications: a literature review to support cost-effective prescribing. Ann Pharmacother 49(6):727–734. doi:10.1177/1060028015574593

Keratoconus and Other Corneal Diseases: Pharmacologic Cross-Linking and Future Therapy

Kimberly M. Hsu and Joel Sugar

Contents

K.M. Hsu • J. Sugar (✉)
University of Illinois at Chicago, Chicago, IL 60612, USA
e-mail: joelsuga@uic.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_23

Abstract

The ability to cross-link collagen fibers and use this technique to strengthen the cornea has become of great interest to ophthalmologists in the last decade. For progressive diseases such as keratoconus, collagen cross-linking confers the possibility of halting progression and stabilizing the cornea, a benefit that is not observed with any other current treatment. Collagen cross-linking uses riboflavin combined with ultraviolet A light to induce the formation of bonds between collagen fibrils that strengthen the cornea. This chapter will discuss the theory, technique, indications, and complications of corneal cross-linking. Much of what will be discussed is in areas of active research that will likely be further clarified as more experience is gained with this procedure.

Keywords

Collagen cross-linking • Cornea • Keratoconus

1 Collagen Structure

Collagen is an important structural protein in mammals. Twenty-eight different types of collagen have been identified in vertebrates, and in humans, collagen accounts for one-third of total body protein. All collagens are comprised of three parallel polypeptide strands in a left-handed, polyproline II-type helical conformation with an offset of one amino acid between strands to form a right-handed triple helix. In this helix, every third amino acid must be glycine. These individual collagen triple helices are known as tropocollagen and ultimately assemble into macromolecular fibers that are found in tissues and bones in the body. As the structure of type I collagen has been elucidated, it has been recognized that microfibrils not only organize together to form fibrils but also interdigitate and cross-link, thereby increasing resistance to separation (Shoulders and Raines 2009).

2 Corneal Structure

The cornea is the transparent "window" of the eye, the anterior structure that provides both structural integrity and optical power to the eye. The adult cornea measures approximately 10–12 mm in diameter and is approximately 0.55 mm thick centrally, slightly increasing in thickness toward the periphery. The cornea provides approximately two-thirds of the refractive power of the eye (with the remaining one-third from the lens). The refractive power of the cornea is determined by the refractive index (1.376) and the radius of curvature of the cornea. Normally, this measures about +44 diopters (Freegard 1997; Nishida and Saika 2011).

The cornea is comprised of five layers: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium (Fig. 1). The corneal

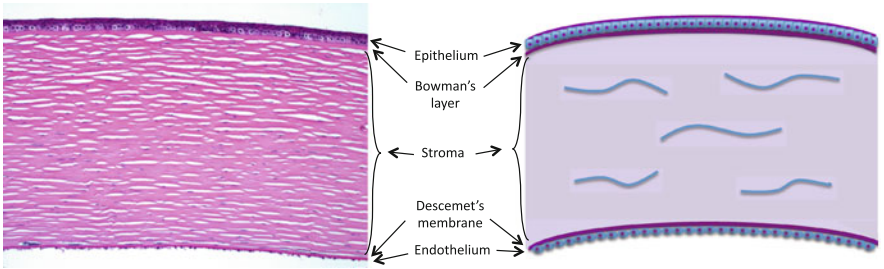

Fig. 1 Layers of the cornea. Cross section of the cornea with PAS stain (*left*, courtesy of Amy Lin, MD) with corresponding schematic (*right*)

epithelium is a layer of nonkeratinized, stratified, squamous epithelial cells serving as a barrier on the ocular surface. It is covered by the tear film, which helps to maintain a smooth epithelial surface and prevent dehydration. Bowman's layer is an acellular layer of collagen fibers and proteoglycans between the epithelium and the rest of the stroma. The largest portion of the cornea is the stroma, which accounts for approximately 90% of the corneal thickness. Maintenance of stromal integrity is crucial to the strength, shape, and transparency of the cornea. The stroma is comprised mostly of collagen, mainly type I although other collagens are represented, and proteoglycans. Collagen fibrils are approximately 35 nm in diameter and are associated with proteoglycans that create uniform spacing of collagen fibrils. Collagen fibers are then arranged in organized parallel rows termed lamellae. Corneal lamellae are interwoven in orthogonal layers in the stroma, and they are thinner and more closely interwoven in the anterior stroma as compared to the posterior stroma. The anterior stroma also provides more structural support than the posterior stroma. Approximately 200–400 lamellae constitute the corneal stroma (Nakayasu et al. 1986; Holmes et al. 2001; Hovakimyan et al. 2012; Sorkin and Varssano 2014). The homogeneity of the diameter of collagen fibers and the distance between them ensures optical clarity. If this regular ordering of collagen is disrupted in conditions such as fibrosis or edema, light is scattered more than usual and the cornea loses its transparency. Keratocytes are the major cellular component of the stroma, but comprise only 2–3% of the total volume. Descemet's membrane is the basement membrane of the corneal endothelium and is composed mostly of type IV collagen. Ruptures of Descemet's membrane can allow aqueous humor into the corneal stroma resulting in corneal edema. The endothelium is a single layer of cells on the posterior surface of the cornea. Endothelial cells pump water from the stroma and are therefore important in maintaining the hydration status of the cornea at 78% water. Human corneal endothelial cells are not able to regenerate to any significant capacity; therefore loss of endothelial cells can lead to corneal edema. Corneal clarity thus requires a smooth anterior surface with adequate coverage by the tear film, regular arrangement of collagen fibers in the stroma, and a functional endothelium that is able to regulate corneal hydration. The cornea also forms a physical barrier between the ocular contents and the

outside world (Nishida and Saika 2011). A number of ocular diseases can lead to corneal alterations and visual deficits. Collagen cross-linking has been recently added to the therapeutic armamentarium for a number of these diseases, and these will be discussed later in the chapter.

3 Collagen Cross-Linking

Cross-linking is the creation of intra- or intermolecular covalent or ionic bonds that connect one polymer chain to another, thereby increasing stability. Collagen cross-linking both occurs naturally and has also been induced in a variety of applications to increase the strength of an end product. Cross-linking can occur in several different pathways:

1. Enyzmatic cross-linking that occurs naturally during the maturation of collagen fibrils. Collagen fibrils have short segments at the ends of the collagen chain (termed telopeptides) that do not assume the triple-helical conformation. The lysine and hydroxylysine residues in these end chains react to form cross-links via the enzyme lysyl oxidase. Lysyl oxidase induces aldehyde formation via oxidative deamination of the lysine and hydroxylysine residues, and these aldehydes react with neighboring aldehyde groups in an aldol condensation reaction or with ε-amino groups of amino acids to create covalent cross-links. Deficiency of lysyl oxidase is clinically relevant in certain types of Ehlers–Danlos syndrome. In addition, decreased distribution of lysyl oxidase has been shown in keratoconic corneas (Ashwin and McDonnell 2010; Dudakova et al. 2012; Hovakimyan et al. 2012; Raiskup and Spoerl 2013).
2. Nonenzymatic glycation involves the spontaneous formation of a bond between a reducing sugar and the amino group of a protein. Additional reactions lead to cross-links termed advanced glycation end products (AGEs) such as pentosidine. This reaction occurs naturally during aging and is accelerated in diabetics. Increased levels of pentosidine have been found in diabetic corneas compared to age-matched controls. An increase in the cross-sectional area of collagen molecules with age has also been attributed to this increase in glycation. This is the basis for the thought that diabetes is protective in keratoconus and that the progression of keratoconus decreases with age (Malik et al. 1992; Sady et al. 1995; Elsheikh et al. 2007; Ashwin and McDonnell 2010).
3. Chemical cross-linking involves the use of a chemical that binds to collagen and results in the formation of bonds between collagen molecules. Formaldehyde, glutaraldehyde, and genipin are examples that have been used. These agents are used in tissue fixation and also to increase durability of bioprosthetics, such as in artificial heart valves (Ashwin and McDonnell 2010; Dunn 2012).
4. Photooxidative cross-linking, more specifically involving riboflavin with ultraviolet (UV) A, has been recently of interest for inducing collagen cross-links in corneal tissue. Riboflavin (vitamin B2) is a nontoxic, essential constituent of cells and plays a critical role in normal cellular metabolism as the precursor to flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). During

collagen cross-linking, riboflavin increases the absorption of UVA light in the corneal stroma from approximately 30 to 95%, thereby serving two roles: increasing the generation of radicals induced by UV light and protecting the deeper structures of the eye from the damaging effects of UV light (Spoerl et al. 1998; Spoerl and Seiler 1999). 370 nm was chosen to be used clinically as it is the absorptive peak of riboflavin. UVA excites riboflavin into its singlet and triplet states. Two different reactions then take place, depending on the oxygen availability. In a type I reaction, radicals are formed in a low-oxygen environment. In a type II reaction, oxygen reacts with riboflavin to produce singlet molecular oxygen. These highly reactive molecules then induce covalent bonds between collagens, proteoglycans, and nucleic acids. The exact nature and location of these cross-links is still an area of active research. At high concentrations, riboflavin is a radical scavenger; thus increasing riboflavin results in a state of saturation rather than increased radical formation (Hovakimyan et al. 2012; Kamaev et al. 2012; Meek and Hayes 2013; Raiskup and Spoerl 2013).

3.1 History of Corneal Collagen Cross-Linking

In 1998, Spoerl et al. published results from a cross-linking study in porcine eyes. Eight groups were treated with one of the following: UV light (254 nm) alone, 0.5% riboflavin alone, 0.5% riboflavin and UV light (365 nm), 0.5% riboflavin and blue light (436 nm), 0.5% riboflavin and sunlight, glutaraldehyde (1% or 0.1%), or Karnovsky's solution 0.1% (paraformaldehyde, sodium hydroxide, and glutaraldehyde). Riboflavin and UV light, glutaraldehyde, and Karnovsky's solution were found to have significant stiffening effect on the cornea (Fig. 2) (Spoerl et al. 1998). A pilot trial with riboflavin and UVA in humans was started in 1998, and the results were published in 2003 by Wollensak et al. In this study, 22 patients with progressing keratoconus were treated with riboflavin drops after epithelial debridement and exposed to UVA (370 nm, 3 mW/cm^2) at 1 cm for 30 min. Progression was stopped in all patients, and 70% of eyes had reduction of keratometry readings (flattening of the cornea) and refractive error. 65% of patients had improved visual acuity (Wollensak et al. 2003a).

3.2 Standard "Dresden" Protocol

The standard treatment protocol is commonly referred to as the "Dresden protocol," based on the original description by Wollensak et al. from the Technical University of Dresden (2003a, 2003a, b, c). It includes the following steps:

- Application of topical anesthetic
- Removal of the central 7–9 mm of the corneal epithelium to ensure riboflavin penetration into the stroma

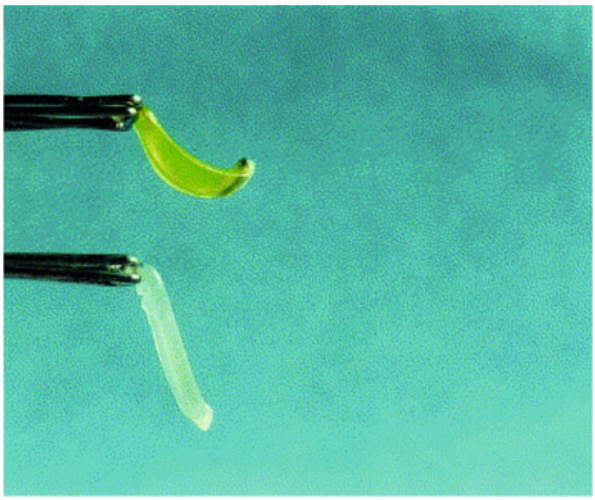

Fig. 2 Stiffening effect of porcine corneas after cross-linking. The treated cornea (*above*) is able to maintain its curvature while the untreated cornea (*below*) is not. Reproduced with permission from Wollensak et al. (2003b)

- Application of riboflavin 0.1% solution in 20% dextran every 5 min for 30 min to achieve adequate penetration through stroma
- Exposure to UVA radiation (370 nm, 3 mW/cm^2) for 30 min with application of riboflavin every 5 min
- Placement of bandage contact lens to promote re-epithelialization of the corneal surface

Minor variations include the duration of the riboflavin application prior to treatment and the application of pilocarpine to constrict the pupil preoperatively to reduce UVA penetration to the lens and posterior segment (Sorkin and Varssano 2014).

3.3 Biomechanics

Wollensak demonstrated in an ex vivo experiment that corneal rigidity is increased after cross-linking. Stress-strain measurements revealed a rise in stress of 71.9% in porcine corneas and 328.9% in human corneas and an increase in Young's modulus by a factor of 1.8 in porcine corneas and 4.5 in human corneas. It is thought that the greater effect in human corneas is due to the relatively thinner tissue and therefore relatively larger portion of the cornea that was subjected to cross-linking (Wollensak et al. 2003a, b, c). In another study, it was demonstrated that increases in stress and Young's modulus persisted over an 8-month period (Wollensak and Iomdina 2009a, b). There is noted to be a depth-dependent stiffening effect whereby the anterior 200 μm of the cornea absorbs up to 70% of UVA irradiation and the subsequent 200 μm absorbs only 20% of UVA irradiation. Thus, the stiffening effect is greater in the anterior cornea as compared to the posterior cornea (Kohlhaas et al. 2006). This is corroborated by testing the thermomechanical

properties of the anterior and posterior cornea, which showed that a higher shrinkage temperature was observed for the anterior cross-linked stroma as compared to the posterior stroma (Spoerl et al. 2004a, b). Transmission electron microscopy of cross-linked collagen fibers showed a 12% increase in the diameter of collagen fibers in the anterior stroma, with a smaller effect in the posterior stroma. This is thought to be due to the induced cross-links that push the collagen polypeptide chains apart, resulting in increased intermolecular spacing and subsequently thicker appearing fibers (Wollensak et al. 2004b). Collagen cross-linking may also have a stabilizing biochemical effect; it has been reported that cross-linking increases corneal resistance to digestion by enzymes such as pepsin, trypsin, and collagenase. It is postulated that this is due to changes in the tertiary structure of collagen that inhibits the ability of these enzymes to reach their target sites (Spoerl et al. 2004a, b).

3.4 Cellular Changes in Corneal Cross-Linking

In the standard protocol, the epithelium is removed and usually heals in several days. Three to six months may be required to regain normal epithelial thickness. Apoptosis of keratocytes occurs in the anterior 300 μm of the stroma (Wollensak et al. 2004a; Wollensak 2010; Mazzotta et al. 2012). This is accompanied by lacunar edema. Over the course of several weeks, new keratocytes begin to migrate in from the periphery and repopulate the stroma (Mencucci et al. 2010). Using in vivo confocal microscopy, the stroma is noted to be hyporeflective in the first few months after cross-linking due to loss of keratocytes and stromal edema; after 6 months the stroma becomes hyperreflective due to increased keratocytes and collagen compaction (Mazzotta et al. 2008). The subepithelial nerve plexus disappears after cross-linking, and is observed to begin to regenerate after 7 days, although full recovery may take 6–12 months (Mazzotta et al. 2008; Xia et al. 2011).

4 Variations from the Standard Protocol

4.1 "Epi-On" Cross-Linking

Riboflavin is a hydrophilic macromolecule that does not easily traverse past the tight junctions between epithelial cells. Therefore, the standard "epi-off" protocol for cross-linking requires the removal of the epithelium to facilitate the penetration of riboflavin into the stroma. However, there has been recent interest and debate in cross-linking without the removal of the epithelium ("epi-on" or "trans-epithelial" cross-linking). This confers the advantages of less postoperative pain and decreased risk of infection.

Cross-linking without the removal of the epithelium confers significantly less stiffening of the tissue due to decreased penetration of riboflavin (Tao et al. 2013).

Confocal microscopy studies showed stromal cellular changes in epithelium-off cross-linking and no significant changes in trans-epithelial cross-linking (Mastropasqua et al. 2013). Success in trans-epithelial cross-linking requires some alteration of the epithelium to enhance permeability. One method is through the use of a chemical agent to loosen epithelial tight junctions, such as through the use of benzalkonium chloride (BAC) or ethylenediaminetetraacetic acid (EDTA) (Kissner et al. 2010; Raiskup et al. 2012). Several studies have demonstrated the efficacy of such methods, although the increase in Young's modulus was only one-fifth of that in epithelium-off cross-linking in one study (Wollensak and Iomdina 2009a, b), and clinical studies in patients have generally found a significant but limited response when the epithelium is left intact (Leccisotti and Islam 2010; Filippello et al. 2012; Stojanovic et al. 2012). These methods may be especially useful in pediatric and uncooperative patients. It is not known how much of a stiffening effect is required for optimal treatment; long-term efficacy will need to be assessed by further studies.

A number of methods have been suggested to increase the penetration of riboflavin in epi-on cross-linking. Iontophoresis, by applying a small current, can help deliver the negatively charged riboflavin into the stroma and has been used with some benefit (Bikbova and Bikbov 2014). Sparing some of the epithelium by removing patches in a grid-like pattern has also been advocated, although with disappointing results with studies showing limited and inhomogeneous penetration of riboflavin using this method (Samaras et al. 2009; Malhotra et al. 2012). The creation of a corneal pocket and subsequent instillation of riboflavin into the pocket has been suggested (Daxer et al. 2010). The development of riboflavin nanoemulsions that increase diffusion across the epithelium provides an additional option (Bottos et al. 2013).

4.2 Accelerated Cross-Linking

Standard cross-linking uses a 3 mW/cm^2 irradiance from a 370 nm light source for 30 min for a cumulative dose of 5.4 J/cm^2. Accelerated cross-linking is based on the Bunsen–Roscoe reciprocity law, where higher intensity and lower exposure time can lead to the same cumulative dose (Bunsen and Roscoe 1862). Initial studies in porcine eyes showed equivalent stress-strain measurements between the standard treatment and an accelerated treatment using 10 mW/cm^2 for 9 min (Schumacher et al. 2011). As type II reactions in cross-linking require oxygen, there is concern that oxygen availability will be a limiting factor in shorter treatment times, leading to decreased efficacy (Richoz et al. 2013a). There is also a concern that the higher fluency will lead to greater radiation toxicity. Increased keratocyte apoptosis was seen in one study following accelerated cross-linking (Touboul et al. 2012). The risk of endothelial cell loss has not been observed to be increased in clinical studies (Kanellopoulos 2012; Gatzioufas et al. 2013). When the cornea is treated with standard cross-linking, a demarcation line is seen at approximately 300–350 μm, indicating the posterior extent of treatment (Mazzotta et al. 2008). In accelerated

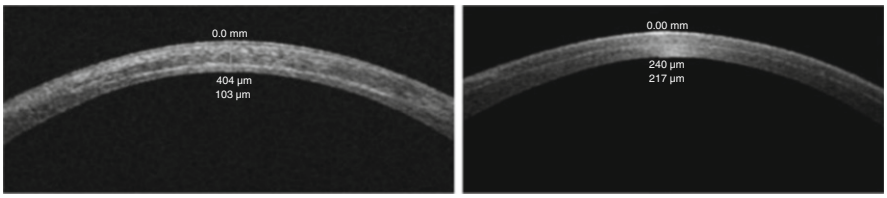

Fig. 3 Optical coherence tomography of the cornea after standard cross-linking (*left*) and accelerated cross-linking (*right*), with the demarcation line much more anterior in the latter. Reproduced with permission from Kymionis et al. (2014a)

cross-linking, it has been reported that the demarcation line is more anterior (Touboul et al. 2012; Kymionis et al. 2014a, b); therefore the treatment depth is more shallow in accelerated cross-linking (Fig. 3). However, a recent study with a modified accelerated protocol using 9 mW/cm^2 for 14 min showed a similar demarcation line depth between the modified accelerated protocol and the standard protocol (Kymionis et al. 2014a, b). Clinical trials using accelerated cross-linking protocols are an area of active research; studies in mild–moderate keratoconus patients have shown stabilization of the cornea for up to 1 year. Of note, the treatment fluence and duration are variable between studies, with some using an accelerated treatment protocol of 30 mW/cm^2 for 3–4 min (Elbaz et al. 2014; Sherif 2014; Tomita et al. 2014). Pulsed UVA light instead of continuous UVA light is also being studied (Mazzotta et al. 2014). More long-term trials are needed to assess the efficacy and safety of accelerated cross-linking (MacGregor et al. 2014; Tsatsos et al. 2014).

4.3 Collagen Cross-Linking in Thin Corneas

Corneas thinner than 400 µm are generally excluded from the standard treatment due to concern regarding endothelial damage. A cytotoxic level of 0.65 J/cm^2 of UVA light was found to reach the endothelium in corneas thinner than 400 µm (Wollensak et al. 2003a, b, c). However, patients with keratoconus or other ectatic disorders may often have thin corneas, and patients should have 400 µm thickness after epithelial removal. Moreover, it has been shown that additional thinning of 75–87 µm can take place during the course of the procedure, possibly due to evaporative effect or oncotic effect of the 20% dextran in the riboflavin solution (Kymionis et al. 2009c; Holopainen and Krootila 2011). Indeed, in a study with patients with corneal thickness less than 400 µm after epithelial removal, standard cross-linking treatment led to significant decrease in endothelial cell density (Kymionis et al. 2012).

A hypoosmolar riboflavin solution has been advocated to preoperatively increase the thickness of the cornea and therefore safely proceed with cross-linking in those patients with corneas initially thinner than 400 µm. The hypoosmolar riboflavin 0.1% solution is created by diluting riboflavin-5-phosphate 0.5% in

sodium chloride 0.9% solution, without the use of dextran as is in the isoosmolar version. This was used successfully to stabilize keratoconic patients with a minimum corneal thickness of 323 μm; however a failure was described in a patient with corneal thickness of 268 μm leading to the conclusion that even with this method, patients should have a minimum preoperative stromal thickness of 330 μm (Hafezi et al. 2009; Hafezi 2011). There is also a concern that the swelling effect of the hypoosmotic solution is transient within 10–30 min; therefore the risk exists of thinning during the procedure with the possibility of endothelial damage. Although stabilization of the ectasia is achieved, the effects may be more modest than in standard cross-linking (Padmanabhan and Dave 2013).

A customized epithelial debridement technique has been described in which an island of the epithelium is preserved in the thinnest area, and standard cross-linking was then applied (Kymionis et al. 2009a). Other solutions to thin corneas include using epithelium-on treatment, decreasing the irradiation dose, or methods to decrease riboflavin penetration such that it does not reach the endothelium such as briefer applications or higher concentrations of riboflavin, which in turn increases the toxicity threshold of the endothelium (Raiskup and Spoerl 2013). Most recently, contact lens-assisted collagen cross-linking (CACXL) has been suggested in which a contact lens soaked in riboflavin is used to increase the functional thickness of the cornea (Jacob et al. 2014).

5 Indications for Cross-Linking

5.1 Keratoconus

Keratoconus is characterized by thinning and protrusion of the cornea without obvious inflammation (Fig. 4). This leads to progressive myopia and irregular astigmatism that impairs vision. It is generally a bilateral disease, although it may be asymmetric in presentation. Estimates of prevalence vary between 10 and

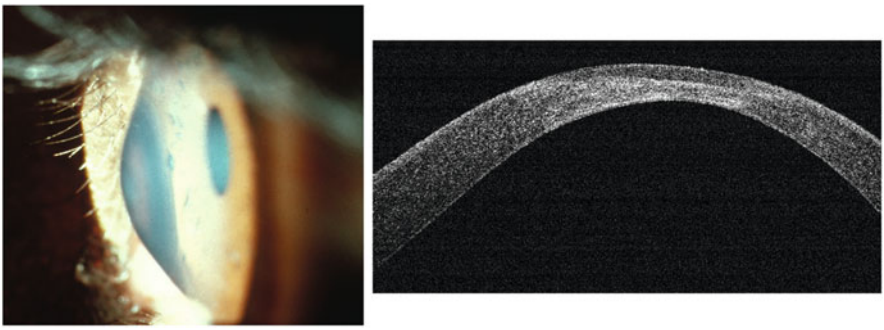

Fig. 4 Clinical photograph of a patient with keratoconus (*left*) and optical coherence tomography of a patient with keratoconus with marked corneal thinning and scarring (*right*, courtesy of Jose de la Cruz, MD)

230 per 100,000 and vary by ethnicity. Corneal changes often begin in puberty and progress until the third or fourth decade of life, when it stabilizes. It has been found that patients with keratoconus have normal-sized collagen fibers, but a number of collagen lamellae are abnormally low in areas of thinning. It has been suggested that weak interlamellar connections cause lamellar slippage, which results in the thinning without collagenolysis seen in keratoconus (Feder and Gan 2011; Vazirani and Basu 2013).

The etiology of keratoconus is not entirely known but is felt to be multifactorial. Most cases are sporadic, although there is good evidence that genetic factors likely play a role in at least some patients. Several studies have estimated a family history in 5–15% of patients. Twin studies have demonstrated high concordance of keratoconus among twins. Family members of patients with keratoconus have been found to be more likely to have corneal changes suspicious for keratoconus. Keratoconus has also been associated with numerous systemic and genetic disorders, including Down syndrome, connective tissue disorders such as Ehlers–Danlos syndrome and osteogenesis imperfecta, and atopic diseases. It is thought that the underlying predisposition in many of these cases is either abnormal collagen elasticity or chronic eye rubbing behavior that leads to eventual corneal ectasia. Other environmental factors that lead to eye rubbing or contact lens wear have also been associated with keratoconus. Lastly, although keratoconus has been conventionally described as a noninflammatory disorder, there is some recent evidence that suggests there may be an imbalance of pro-inflammatory and anti-inflammatory factors in the tear film in patients with keratoconus compared to normal controls. Thus, the etiology of keratoconus remains complex and multifactorial, and many favor a "two-hit" hypothesis wherein a genetic predisposition combined with an environmental insult such as eye rubbing ultimately leads to disease manifestation (Sugar and Macsai 2012; Chang and Chodosh 2013).

Patients with keratoconus often present in the teenage to young adult years with blurred vision and frequent need for glasses prescription change due to changes in corneal curvature. Diagnosis is made by clinical signs and findings of irregular astigmatism. The gross conical protrusion of the cornea may be observed in several different ways. Munson's sign is a V-shaped indentation in the lower eyelid when the patient looks down due to protrusion of the cone. Rizzuti's sign is the finding of a conical reflection on the nasal cornea when a light is shined from the temporal side. The oil droplet or "Charleaux" sign is the finding of a dark oil droplet shape in retroillumination when the pupil is dilated. Slit lamp examination reveals corneal ectasia and thinning at the apex of the cone, which is often located in the inferior paracentral cornea. This is often accompanied by other signs such as the following: Vogt's striae which are parallel vertical wrinkles in the posterior stroma, Fleischer's ring which is an iron line at the base of the cone, or corneal scarring due to breaks in Bowman's layer. Patients can also present with acute hydrops, in which a sudden break in Descemet's membrane results in corneal edema (Feder and Gan 2011; Vazirani and Basu 2013).

The astigmatism in keratoconus can be measured using manual keratometry, corneal topography, or corneal tomography. Criteria for screening for patients with

keratoconus have been developed using topographic indices. Rabinowitz suggested four indices for screening: central corneal power greater than 47.2 diopters, inferior–superior dioptric asymmetric greater than 1.2, simulated keratometry value astigmatism greater than 1.5 diopters, and skewed radial axes greater than 21° (Rabinowitz 1995).

The management of keratoconus usually follows a stepwise approach as disease severity increases. Early changes in refractive error can be adequately corrected with glasses. If glasses fail to provide reasonable vision, contact lenses are then used. In particular, rigid gas permeable contact lenses are able to mask some amount of irregular astigmatism by creating a new anterior refractive surface. If both glasses and contact lenses are unable to adequately correct vision, traditionally, a corneal transplant may be required. This may be in the form of either a deep anterior lamellar keratoplasty or a full thickness penetrating keratoplasty. Recently, new advances have begun to popularize the use of intracorneal ring segment insertion or collagen cross-linking in these patients before they reach the need for corneal transplantation. Intracorneal crescentic acrylic ring segments are inserted into the corneal stroma to flatten the central cornea and improve vision and contact lens fit (Feder and Gan 2011; Vazirani and Basu 2013). Corneal cross-linking will be discussed below. An even newer proposal is the transplantation of a Bowman layer graft to reduce corneal ectasia and postpone more invasive procedures (van Dijk et al. 2014).

Collagen cross-linking is a unique treatment for keratoconus in that the goal of treatment is to stop the progression of the ectasia. Since the publication of the first human trial in 2003 (Wollensak et al. 2003a, b, c), there have been an increasing number of reports advocating the safety and efficacy of collagen cross-linking in patients with keratoconus. Many of these studies demonstrate 1–2 diopters of flattening of keratometry values, which appeared stable over the first few years after cross-linking. Some groups report small but significant improvements in visual acuity (Raiskup-Wolf et al. 2008; Caporossi et al. 2010; Asri et al. 2011; Hersh et al. 2011; O'Brart et al. 2011, 2013; Goldich et al. 2012; Vinciguerra et al. 2013). Large, longitudinal randomized controlled trials are lacking. Recently, the 3-year results of a randomized controlled trial were published. This Australian study recruited 100 participants with progressive keratoconus to receive the standard cross-linking protocol. After 3 years, the maximum keratometry (Kmax) of the control group had increased by a mean of +1.75 diopters, whereas the treated group had flattened by a mean of −1.03 diopters. Uncorrected visual acuity increased by −0.15 logMAR and best corrected visual acuity by −0.09 logMAR in treated patients (Wittig-Silva et al. 2014). Thus the progression of keratoconus appears to be halted and the corneal steepening mildly reversed.

5.2 Pellucid Marginal Corneal Degeneration

Pellucid marginal corneal degeneration is an ectatic disorder with thinning generally more peripheral and inferior that in keratoconus. There is a classic "beer belly"

appearance with maximal corneal protrusion above the area of thinning. As in keratoconus, patients also have high astigmatism. The use of collagen cross-linking has been reported in these patients, with inferiorly decentered irradiation to treat the area of thinning, with benefit similar to that seen in keratoconus patients (Spadea 2010; Hassan et al. 2014; Moshirfar et al. 2014). Animal studies suggest that the decentered irradiation involving the limbus does not affect the regenerative capacity of limbal stem cells (Richoz et al. 2014).

5.3 Post-LASIK Ectasia

Laser in situ keratomileusis (LASIK) is a popular procedure performed to correct refractive error. It involves creating a flap of approximately 110 μm with a microkeratome or femtosecond laser, raising the flap, applying the treatment to the stromal bed with an excimer laser, and replacing the flap. According to the Munnerlyn formula, the depth of ablation in micrometers is equal to the square of the diameter of the optical ablation zone (in millimeters) multiplied by the dioptric correction divided by 3. Thus, ablation depth is increased as diopters of correction increases and optical zone increases. Additionally the cut LASIK flap, although replaced, does not provide structural support postoperatively (Munnerlyn et al. 1988; Sutton et al. 2014).

Post-LASIK ectasia resembling keratoconus was first described in 1998 by Seiler et al. (1998). Progressive steepening of the cornea results in increased myopia and astigmatism, with decrease in visual acuity. As LASIK removes the corneal tissue and the flap does not provide structural support as described above, the strength of the cornea post-LASIK is dependent on the residual stromal bed. Additionally, the anterior stroma in normal corneas provides more tensile strength than the posterior cornea. It is thought that the residual stromal thickness should be at least 250–300 μm posttreatment to reduce the risk of ectasia. It follows then that patients with high myopia and a deeper ablation depth would be at higher risk for ectasia. Corneal topography should be examined carefully prior to the procedure for any signs of abnormality that may suggest a tendency to developing corneal ectasias such as keratoconus; if any are found, LASIK is not recommended. Additionally, since keratoconus often manifests in the teens to 20s, younger patients are at higher risk for ectasia as they may have an underlying tendency that has not yet manifested (Randleman 2006; Cheema et al. 2012).

Traditional management of post-LASIK ectasia is similar to keratoconus: glasses and rigid gas permeable contact lenses are initial steps. In recent years, there have been reports of using collagen cross-linking to stabilize these corneas. Both stabilization and mild improvement in keratometry have been reported, with improvement in vision. This effect may be less than that seen in keratoconus, possibly due to biomechanical differences caused by the presence of a LASIK flap. Collagen cross-linking strengthens the anterior stroma more than the posterior stroma, and in the case of LASIK patients, the flap is the most anterior portion, but does not contribute to mechanical stability. It is also unclear whether diffusion of

riboflavin is affected by the LASIK flap (Hersh et al. 2011; Richoz et al. 2013b; Yildirim et al. 2014).

5.4 Keratitis

Infectious keratitis can lead to significant visual complications including corneal melting and perforation. Recently, there has been interest in using collagen cross-linking as an adjunctive therapy for patients with infectious keratitis. The goal of the treatment would be twofold: (1) to kill the microorganism and (2) to strengthen the cornea and resist proteolysis that could lead to melting and perforation. Bacterial keratitis is most common, and there is in vitro evidence that riboflavin and UVA is effective against bacteria (Martins et al. 2008). Several studies also support the utility of collagen cross-linking in the treatment of patients with advanced bacterial keratitis (Bettis et al. 2012; Price et al. 2012; Alio et al. 2013; Vazirani and Vaddavalli 2013; Shetty et al. 2014b), although the degree of efficacy has yet to be well defined. In one prospective study, cross-linking did not decrease the time to corneal healing, but did decrease corneal perforations and recurrence of infection (Said et al. 2014).

The data regarding efficacy of cross-linking in fungal keratitis is mixed. In vivo studies in animals and case reports suggest that there may be some effect in treating fungi with cross-linking (Galperin et al. 2012; Li et al. 2013). However, a retrospective study comparing patients who received medical therapy alone vs. medical therapy with collagen cross-linking did not find any advantage in terms of recovery, vision, or need for tectonic corneal transplant (Vajpayee et al. 2015). In addition, fungal infections can often be located deep in the cornea; in these cases it would be difficult to adequately treat the infection without potential damage to the endothelium.

The least convincing data is for treatment of *Acanthamoeba* keratitis with cross-linking. *Acanthamoeba* is a protozoa found commonly in freshwater which can cause a keratitis that is difficult to eradicate due to the formation of resistant cysts. Contact lens wearers are at particularly high risk for *Acanthamoeba* infection. Although it has been reported that patients with *Acanthamoeba* keratitis benefitted from cross-linking combined with traditional treatment (Khan et al. 2011; Demirci and Ozdamar 2013), the true benefit is questionable as studies done in vitro and in vivo in animals have failed to show any effect of riboflavin and UVA on *Acanthamoeba* trophozoites or cysts (Kashiwabuchi et al. 2011; del Buey et al. 2012; Berra et al. 2013).

5.5 Bullous Keratopathy

Pseudophakic bullous keratopathy (PBK) occurs due to endothelial cell decompensation after cataract surgery. As the pump function of the endothelial cells fails, corneal edema develops. Epithelial edema is often accompanied by the formation of

bullae on the surface of the eye that may rupture and become painful. Collagen cross-linking has been attempted in these situations in an effort to maintain the compact structure of the cornea, which should lead to improved vision, decreased bullae, and decreased pain. Most studies using collagen cross-linking have focused on PBK, but it has also been applied to patients with other etiologies for corneal edema, such as Fuchs corneal endothelial dystrophy. Several studies have demonstrated early benefit of cross-linking in these patients, but with regression over 3–6 months with increased corneal thickness, bullae reformation, and pain (Cordeiro Barbosa et al. 2010; Ghanem et al. 2010; Arora et al. 2013; Sharma et al. 2014).

5.6 Combination with Other Refractive Procedures

The goal of collagen cross-linking is to stabilize the cornea in ectatic disorders and in many studies there has been minimal improvement in visual acuity. There has therefore been interest in combining collagen cross-linking with refractive surgery to both stabilize the cornea and improve visual outcomes. Photorefractive keratectomy (PRK) uses an excimer laser to ablate and reshape the anterior corneal stroma to correct refractive errors. In early studies combining PRK with cross-linking, PRK was performed months after collagen cross-linking to improve vision (Kanellopoulos and Binder 2007). Subsequently, good results were reported with the "Athens protocol," in which the two procedures are combined, and collagen cross-linking is performed immediately following PRK (Kanellopoulos 2009; Kymionis et al. 2009b; Kanellopoulos and Asimellis 2014). In terms of sequence, it is thought that PRK prior to collagen cross-linking is better as PRK following cross-linking results in the removal of the cross-linked anterior cornea, which may diminish the benefit from the cross-linking procedure. In addition to patients with keratoconus, this has been performed successfully in patients with post-LASIK ectasia (Kanellopoulos and Binder 2011; Kymionis et al. 2011).

Intracorneal ring segments (ICRS) are polymethyl methacrylate semicircular ring segments that are generally placed in pairs within the corneal stroma. They were originally designed to correct myopia by flattening the central cornea. ICRS have been used alone to treat keratoconus, but they do not prevent the progression of ectasia. Therefore, they have been combined with cross-linking in an effort to both flatten and stabilize the cornea. A stromal tunnel is created in which to insert the ICRS, and some have used this tunnel as a method of instilling riboflavin into the corneal stroma without epithelial removal. Various sequences for ICRS implantation and cross-linking have been used, with either being performed first and then the other at a later date or with both procedures performed on the same day. "Triple therapy" with PRK, collagen cross-linking, and ICRS implantation has also been used. Visual outcome and corneal stability seem to be improved with these combinations (Avni-Zauberman and Rootman 2014; Li et al. 2014).

As discussed previously, patients who require high corrections for LASIK may be at increased risk for ectasia. In addition, patients, especially those with hyperopic

correction, may experience regression after the procedure. In an effort to stabilize the cornea after LASIK, collagen cross-linking has been performed prophylactically in at-risk patients. A few studies have suggested that this is safe and effective; however long term data has yet to be seen (Kanellopoulos 2012; Kanellopoulos and Kahn 2012).

6 Complications

6.1 Postoperative Infections

Standard collagen cross-linking involves the creation of an epithelial defect, which is usually then covered with a bandage contact lens. Topical corticosteroids are also commonly used. These factors likely increase the risk of infection after the procedure, and a variety of infections have been reported after cross-linking. Bacterial infections including *Staphylococcus aureus* (Shetty et al. 2014a), *Escherichia coli* (Pollhammer and Cursiefen 2009), *Pseudomonas aeruginosa* (Sharma et al. 2010), *Staphylococcus epidermidis* (Pérez-Santonja et al. 2009), and even polymicrobial infections (Zamora and Males 2009) have been reported. Herpetic ulcers have been observed (Kymionis et al. 2007; Yuksel et al. 2011). More unusual infections including those involving *Microsporidia* (Gautam et al. 2013), *Fusarium* (Garcia-Delpech et al. 2010), and *Acanthamoeba* (Rama et al. 2009) have been encountered. Some patients have also been noted to develop sterile infiltrates that are culture negative (Mangioris et al. 2010; Lam et al. 2014) but improved with topical antibiotics and steroids.

6.2 Corneal Haze

Stromal haze is commonly noted after cross-linking. In one study, it was noted that the haze peaked at 1 month, plateaued until 3 months, and then significantly decreased at 6 and 12 months (Greenstein et al. 2010). Although it seems that most patients will develop a temporary haze that improves and is not visually significant, some patients may develop a late-onset permanent corneal haze or scarring that may impair vision due to the opacity or due to increased astigmatism. It has been suggested that higher keratometry values and lower corneal thickness are risk factors; however significant haze has been reported in patients who had only mild keratoconus as well (Mazzotta et al. 2007; Raiskup et al. 2009; Lim et al. 2011). Compared to haze that is sometimes seen after photorefractive keratectomy, the haze after cross-linking appears to extend deeper into the anterior stroma and be more reticular in nature. This is likely associated with the depth of treatment and keratocyte loss (Dhawan et al. 2011).

6.3 Endothelial Damage

As discussed previously, a cytotoxic level of 0.65 J/cm^2 of UVA light was found to reach the endothelium in corneas thinner than 400 μm (Wollensak et al. 2003a, b, c). With careful attention to using the recommended protocols, the risk to endothelial cells appears to be small (Wittig-Silva et al. 2014). However, the rare complication of corneal edema due to endothelial failure has been reported and in some cases has led to the necessity for corneal transplantation (Bagga et al. 2012; Sharma et al. 2012).

6.4 Treatment Failures

Treatment is usually considered successful in collagen cross-linking if the cornea steepening is stabilized. The failure rate (i.e., percentage of eyes with progression) in one study was 7.6%. This is associated with a high preoperative keratometry reading of >58 diopters. 2.9% of eyes lost two or more lines of vision in this study. Age greater than 35 years and a preoperative vision of better than 20/25 were associated with higher complication risk (Koller et al. 2009).

7 Other Methods of Cross-Linking

Although riboflavin with UVA has become increasing popular over the last 10 years in corneal cross-linking, there are many other options for cross-linking that may also prove to be useful in the future. Some of these are chemical cross-linkers, which are simpler to administer as it is a single agent and without the possibility of radiation toxicity from UVA. Tissue fixatives such as formaldehyde, glutaraldehyde, and Karnovsky's solution are well-known cross-linkers but are also significantly toxic in vivo, thus not used (Sorkin and Varssano 2014). Genipin is a natural compound found in the fruit of *Gardenia jasminoides*. It has been tested on porcine corneas and found to significantly increase the stiffness of the corneas as well as increase resistance to collagenase (Avila and Navia 2010). In an animal study comparing genipin to riboflavin and UVA, rigidity was similar between the two and there was minimal toxicity to endothelial cells (Avila et al. 2012). Another naturally occurring cross-linker is proanthocyanidin, which is found in grape seeds (Han et al. 2003). Beta-nitro alcohols have been studied in vitro and found to be potent cross-linkers, perhaps even more so than riboflavin and UVA (Paik et al. 2009). They are thought to be effective by functioning as formaldehyde and nitrite donors to cross-link tissue, and preliminary studies suggest that they may be safe in regard to the endothelium (Paik et al. 2010).

An interesting alternative to riboflavin–UVA cross-linking includes that of Rose Bengal and green light. An in vitro study demonstrated that Rose Bengal penetrates approximately 100 μm into the anterior stroma and can be activated by green light (532 nm) to induce cross-linking. Of note, this treatment did not induce the

keratocyte apoptosis that is seen with riboflavin and UVA treatment. Due to the more shallow penetration of Rose Bengal, the cross-linking effect is more anterior in the stroma; however, this may be beneficial in patients with thin corneas who would otherwise be excluded from riboflavin and UVA cross-linking (Cherfan et al. 2013). Another possibility that has been studied in animal models is that of palladium bacteriochlorin 13′-(2-sulfoethyl)amide dipotassium salt (WST11) and near-infrared (NIR) illumination. This treatment significantly increased corneal stiffness, also demonstrated a reduction in keratocytes in the stroma, and is thought to generate hydroxyl and superoxide radicals, but without singlet oxygen as in riboflavin and UVA treatment (Marcovich et al. 2012).

The improved ability to localize treatment, especially in patients with thin corneas who are at risk for endothelial damage with conventional riboflavin and UVA treatment, has prompted the use of focused femtosecond laser light to induce cross-linking. In one study, riboflavin was activated by a femtosecond laser tuned to 760 nm, and this was able to provide localized treatments to stiffen collagen in hydrogels (Chai et al. 2013).

8 FDA Approval

As of the writing of this chapter, collagen cross-linking is not FDA approved in the United States. The FDA granted orphan drug status to the riboflavin ophthalmic solution and cross-linking system in 2011, but the new drug application is still under review. Clinical trials are ongoing in the United States, and this procedure has been performed internationally where approval has been granted.

References

Alio J, Abbouda A, Valle DD, Del Castillo JB, Fernandez JG (2013) Corneal cross linking and infectious keratitis: a systematic review with a meta-analysis of reported cases. J Ophthalmic Inflamm Infect 3(1):47

Arora R, Manudhane A, Saran RK, Goyal J, Goyal G, Gupta D (2013) Role of corneal collagen cross-linking in pseudophakic bullous keratopathy: a clinicopathological study. Ophthalmology 120(12):2413–2418

Ashwin PT, McDonnell PJ (2010) Collagen cross-linkage: a comprehensive review and directions for future research. Br J Ophthalmol 94(8):965–970

Asri D, Touboul D, Fournié P, Malet F, Garra C, Gallois A, Malecaze F, Colin J (2011) Corneal collagen crosslinking in progressive keratoconus: multicenter results from the French National Reference Center for Keratoconus. J Cataract Refract Surg 37(12):2137–2143

Avila M, Navia J (2010) Effect of genipin collagen crosslinking on porcine corneas. J Cataract Refract Surg 36(4):659–664

Avila M, Gerena V, Navia J (2012) Corneal crosslinking with genipin, comparison with UV-riboflavin in ex-vivo model. Mol Vis 18:1068–1073

Avni-Zauberman N, Rootman D (2014) Crosslinking and intracorneal ring segments-review of the literature. Eye Contact Lens 40(6):365–370

Bagga B, Pahuja S, Murthy S, Sangwan V (2012) Endothelial failure after collagen cross-linking with riboflavin and UV-A: case report with literature review. Cornea 31(10):1197–1200

Berra M, Galperín G, Boscaro G, Zarate J, Tau J, Chiaradia P, Berra A (2013) Treatment of Acanthamoeba keratitis by corneal cross-linking. Cornea 32(2):174–178
Bettis D, Hsu M, Moshirfar M (2012) Corneal collagen cross-linking for nonectatic disorders: a systematic review. J Refract Surg 28(11):798–807
Bikbova G, Bikbov M (2014) Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin. Acta Ophthalmol 92(1):e30–e34
Bottos K, Oliveira A, Bersanetti P, Nogueira R, Lima-Filho A, Cardillo J, Schor P, Chamon W (2013) Corneal absorption of a new riboflavin-nanostructured system for transepithelial collagen cross-linking. PLoS One 8(6), e66408
Bunsen RW, Roscoe HE (1862) Photochemical researches.--Part V. On the measurement of the chemical action of direct and diffuse sunlight. Proc R Soc Lond 12:306–312
Caporossi A, Mazzotta C, Baiocchi S, Caporossi T (2010) Long-term results of riboflavin ultraviolet A corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. Am J Ophthalmol 149(4):585–593
Chai D, Juhasz T, Brown D, Jester J (2013) Nonlinear optical collagen cross-linking and mechanical stiffening: a possible photodynamic therapeutic approach to treating corneal ectasia. J Biomed Opt 18(3)
Chang H-Y, Chodosh J (2013) The genetics of keratoconus. Semin Ophthalmol 28(5-6):275–280
Cheema A, Mozayan A, Channa P (2012) Corneal collagen crosslinking in refractive surgery. Curr Opin Ophthalmol 23(4):251–256
Cherfan D, Verter E, Melki S, Gisel T, Doyle F, Scarcelli G, Yun S, Redmond R, Kochevar I (2013) Collagen cross-linking using rose bengal and green light to increase corneal stiffness. Invest Ophthalmol Vis Sci 54(5):3426–3433
Cordeiro Barbosa M, Barbosa JB, Hirai F, Hofling-Lima AL (2010) Effect of cross-linking on corneal thickness in patients with corneal edema. Cornea 29(6):613–617
Daxer A, Mahmoud H, Venkateswaran RS (2010) Corneal crosslinking and visual rehabilitation in keratoconus in one session without epithelial debridement: new technique. Cornea 29 (10):1176–1179
del Buey M, Cristóbal J, Casas P, Goñi P, Clavel A, Mínguez E, Lanchares E, García A, Calvo B (2012) Evaluation of in vitro efficacy of combined riboflavin and ultraviolet a for Acanthamoeba isolates. Am J Ophthalmol 153(3):399–404
Demirci G, Ozdamar A (2013) A case of medication-resistant acanthamoeba keratitis treated by corneal crosslinking in Turkey. Case Rep Ophthalmol Med 2013:608253. doi:10.1155/2013/608253, Epub 2013 Dec 22
Dhawan S, Rao K, Natrajan S (2011) Complications of corneal collagen cross-linking. J Ophthalmol 2011:1–5
Dudakova L, Liskova P, Trojek T, Palos M, Kalasova S, Jirsova K (2012) Changes in lysyl oxidase (LOX) distribution and its decreased activity in keratoconus corneas. Exp Eye Res 104:74–81
Dunn R (2012) Cross-linking in biomaterials. Plast Reconstr Surg 130:18S–26S
Elbaz U, Shen C, Lichtinger A, Zauberman N, Goldich Y, Chan C, Slomovic A, Rootman D (2014) Accelerated (9-mW/cm2) corneal collagen crosslinking for keratoconus-A 1-year follow-up. Cornea 33(8):769–773
Elsheikh A, Wang D, Brown M, Rama P, Campanelli M, Pye D (2007) Assessment of corneal biomechanical properties and their variation with age. Curr Eye Res 32(1):11–19
Feder R, Gan T (2011) Noninflammatory ectatic disorders. In: Krachmer J, Mannis M, Holland E (eds) Cornea. Mosby, Philadelphia, pp 865–887
Filippello M, Stagni E, O'Brart D (2012) Transepithelial corneal collagen crosslinking: bilateral study. J Cataract Refract Surg 38(2):283–291
Freegard TJ (1997) The physical basis of transparency of the normal cornea. Eye (Lond) 11 (Pt 4):465–471
Galperin G, Berra M, Tau J, Boscaro G, Zarate J, Berra A (2012) Treatment of fungal keratitis from Fusarium infection by corneal cross-linking. Cornea 31(2):176–180

Garcia-Delpech S, Díaz-Llopis M, Udaondo P, Salom D (2010) Fusarium keratitis 3 weeks after healed corneal cross-linking. J Refract Surg 26(12):994–995

Gatzioufas Z, Richoz O, Brugnoli E, Hafezi F (2013) Safety profile of high-fluence corneal collagen cross-linking for progressive keratoconus: preliminary results from a prospective cohort study. J Refract Surg 29(12):846–848

Gautam, Jhanji V, Satpathy G, Khokhar S, Agarwal T (2013) Microsporidial keratitis after collagen cross-linking. Ocul Immunol Inflamm 21(6):495–497

Ghanem R, Santhiago M, Berti T, Thomaz S, Netto M (2010) Collagen crosslinking with riboflavin and ultraviolet-A in eyes with pseudophakic bullous keratopathy. J Cataract Refract Surg 36(2):273–276

Goldich Y, Marcovich A, Barkana Y, Mandel Y, Hirsh A, Morad Y, Avni I, Zadok D (2012) Clinical and corneal biomechanical changes after collagen cross-linking with riboflavin and UV irradiation in patients with progressive keratoconus: results after 2 years of follow-up. Cornea 31(6):609–614

Greenstein S, Fry K, Bhatt J, Hersh P (2010) Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: scheimpflug and biomicroscopic analysis. J Cataract Refract Surg 36(12):2105–2114

Hafezi F (2011) Limitation of collagen cross-linking with hypoosmolar riboflavin solution: failure in an extremely thin cornea. Cornea 30(8):917–919

Hafezi F, Mrochen M, Iseli HP, Seiler T (2009) Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. J Cataract Refract Surg 35(4):621–624

Han B, Jaurequi J, Tang BW, Nimni M (2003) Proanthocyanidin: a natural crosslinking reagent for stabilizing collagen matrices. J Biomed Mater Res A 65(1):118–124

Hassan Z, Nemeth G, Modis L, Szalai E, Berta A (2014) Collagen cross-linking in the treatment of pellucid marginal degeneration. Indian J Ophthalmol 62(3):367–370

Hersh P, Greenstein S, Fry K (2011) Corneal collagen crosslinking for keratoconus and corneal ectasia: one-year results. J Cataract Refract Surg 37(1):149–160

Holmes D, Gilpin C, Baldock C, Ziese U, Koster A, Kadler K (2001) Corneal collagen fibril structure in three dimensions: structural insights into fibril assembly, mechanical properties, and tissue organization. Proc Natl Acad Sci 98(13):7307–7312

Holopainen J, Krootila K (2011) Transient corneal thinning in eyes undergoing corneal cross-linking. Am J Ophthalmol 152(4):533–536

Hovakimyan M, Guthoff R, Stachs O (2012) Collagen cross-linking: current status and future directions. J Ophthalmol 2012:1–12

Jacob S, Kumar DA, Agarwal A, Basu S, Sinha P, Agarwal A (2014) Contact lens-assisted collagen cross-linking (CACXL): a new technique for cross-linking thin corneas. J Refract Surg 30(6):366–372

Kamaev P, Friedman M, Sherr E, Muller D (2012) Photochemical kinetics of corneal cross-linking with riboflavin. Invest Ophthalmol Vis Sci 53(4):2360–2367

Kanellopoulos AJ (2009) Comparison of sequential vs same-day simultaneous collagen cross-linking and topography-guided PRK for treatment of keratoconus. J Refract Surg 25(9)

Kanellopoulos AJ (2012) Long-term safety and efficacy follow-up of prophylactic higher fluence collagen cross-linking in high myopic laser-assisted in situ keratomileusis. Clin Ophthalmol 6:1125–1130

Kanellopoulos AJ, Asimellis G (2014) Keratoconus management: long-term stability of topography-guided normalization combined with high-fluence CXL stabilization (the athens protocol). J Refract Surg 30(2):88–93

Kanellopoulos J, Binder P (2007) Collagen cross-linking (CCL) with sequential topography-guided PRK: a temporizing alternative for keratoconus to penetrating keratoplasty. Cornea 26(7):891–895

Kanellopoulos AJ, Binder P (2011) Management of corneal ectasia after LASIK with combined, same-day, topography-guided partial transepithelial PRK and collagen cross-linking: the athens protocol. J Refract Surg 27(5):323–331

Kanellopoulos AJ, Kahn J (2012) Topography-guided hyperopic LASIK with and without high irradiance collagen cross-linking: initial comparative clinical findings in a contralateral eye study of 34 consecutive patients. J Refract Surg 28(11 Suppl):S837–S840

Kashiwabuchi R, Carvalho F, Khan Y, de Freitas D, Foronda A, Hirai F, Campos M, McDonnell P (2011) Assessing efficacy of combined riboflavin and UV-A light (365 nm) treatment of Acanthamoeba trophozoites. Invest Ophthalmol Vis Sci 52(13):9333–9338

Khan Y, Kashiwabuchi R, Martins S, Castro-Combs J, Kalyani S, Stanley P, Flikier D, Behrens A (2011) Riboflavin and ultraviolet light a therapy as an adjuvant treatment for medically refractive Acanthamoeba keratitis. Ophthalmology 118(2):324–331

Kissner A, Spoerl E, Jung R, Spekl K, Pillunat L, Raiskup F (2010) Pharmacological modification of the epithelial permeability by benzalkonium chloride in UVA/Riboflavin corneal collagen cross-linking. Curr Eye Res 35(8):715–721

Kohlhaas M, Spoerl E, Schilde T, Unger G, Wittig C, Pillunat L (2006) Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract Refract Surg 32(2):279–283

Koller T, Mrochen M, Seiler T (2009) Complication and failure rates after corneal crosslinking. J Cataract Refract Surg 35(8):1358–1362

Kymionis G, Portaliou D, Bouzoukis D, Suh L, Pallikaris A, Markomanolakis M, Yoo S (2007) Herpetic keratitis with iritis after corneal crosslinking with riboflavin and ultraviolet A for keratoconus. J Cataract Refract Surg 33(11):1982–1984

Kymionis G, Diakonis V, Coskunseven E, Jankov M, Yoo S, Pallikaris I (2009a) Customized pachymetric guided epithelial debridement for corneal collagen cross linking. BMC Ophthalmol 9:10

Kymionis G, Kontadakis G, Kounis G, Portaliou D, Karavitaki A, Magarakis M, Yoo S, Pallikaris I (2009b) Simultaneous topography-guided PRK followed by corneal collagen cross-linking for keratoconus. J Refract Surg 25(9):S807–S811

Kymionis G, Kounis G, Portaliou D, Grentzelos M, Karavitaki A, Coskunseven E, Jankov M, Pallikaris I (2009c) Intraoperative pachymetric measurements during corneal collagen cross-linking with riboflavin and ultraviolet A irradiation. Ophthalmology 116(12):2336–2339

Kymionis G, Portaliou D, Diakonis V, Karavitaki A, Panagopoulou S, Jankov Ii M, Coskunseven E (2011) Management of post laser in situ keratomileusis ectasia with simultaneous topography guided photorefractive keratectomy and collagen cross-linking. Open Ophthalmol J 5:11–13

Kymionis G, Portaliou D, Diakonis V, Kounis G, Panagopoulou S, Grentzelos M (2012) Corneal collagen cross-linking with riboflavin and ultraviolet-A irradiation in patients with thin corneas. Am J Ophthalmol 153(1):24–28

Kymionis G, Tsoulnaras K, Grentzelos M, Liakopoulos D, Tsakalis N, Blazaki S, Paraskevopoulos T, Tsilimbaris M (2014a) Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. Am J Ophthalmol 158(4):671–675.e1

Kymionis G, Tsoulnaras K, Grentzelos M, Plaka A, Mikropoulos D, Liakopoulos D, Tsakalis N, Pallikaris I (2014b) Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. J Cataract Refract Surg 40(5):736–740

Lam FC, Geourgoudis P, Nanavaty MA, Khan S, Lake D (2014) Sterile keratitis after combined riboflavin-UVA corneal collagen cross-linking for keratoconus. Eye (Lond) 28(11):1297–1303

Leccisotti A, Islam T (2010) Transepithelial corneal collagen cross-linking in keratoconus. J Refract Surg 26(12):942–948

Li Z, Jhanji V, Tao X, Yu H, Chen W, Mu G (2013) Riboflavin/ultraviolet light-mediated crosslinking for fungal keratitis. Br J Ophthalmol 97(5):669–671

Li N, Peng X-J, Fan Z-J (2014) Progress of corneal collagen cross-linking combined with refractive surgery. Int J Ophthalmol 7(1):157–162

Lim L, Beuerman R, Lim L, Tan D (2011) Late-onset deep stromal scarring after riboflavin-UV-A corneal collagen cross-linking for mild keratoconus. Arch Ophthalmol 129(3):360–362
MacGregor C, Tsatsos M, Hossain P (2014) Is accelerated corneal collagen cross-linking for keratoconus the way forward? No. Eye 28(7):786–787
Malhotra C, Shetty R, Kumar R, Veluri H, Nagaraj H, Shetty B (2012) In vivo imaging of riboflavin penetration during collagen cross-linking with hand-held spectral domain optical coherence tomography. J Refract Surg 28(11):776–780
Malik NS, Moss SJ, Ahmed N, Furth AJ, Wall RS, Meek KM (1992) Ageing of the human corneal stroma: structural and biochemical changes. Biochim Biophys Acta 1138(3):222–228
Mangioris G, Papadopoulou D, Balidis M, Poulas J, Papadopoulos NT, Seiler T (2010) Corneal infiltrates after corneal collagen cross-linking. J Refract Surg 26(8):609–611
Marcovich A, Brandis A, Daphna O, Feine I, Pinkas I, Goldschmidt R, Kalchenko V, Berkutzki T, Wagner D, Salomon Y, Scherz A (2012) Stiffening of rabbit corneas by the bacteriochlorophyll derivative WST11 using near infrared light. Invest Ophthalmol Vis Sci 53(10):6378–6388
Martins SA, Combs JC, Noguera G, Camacho W, Wittmann P, Walther R, Cano M, Dick J, Behrens A (2008) Antimicrobial efficacy of riboflavin/UVA combination (365 nm) in vitro for bacterial and fungal isolates: a potential new treatment for infectious keratitis. Invest Ophthalmol Vis Sci 49(8):3402–3408
Mastropasqua L, Nubile M, Lanzini M, Calienno R, Mastropasqua R, Agnifili L, Toto L (2013) Morphological modification of the cornea after standard and transepithelial corneal cross-linking as imaged by anterior segment optical coherence tomography and laser scanning in vivo confocal microscopy. Cornea 32(6):855–861
Mazzotta C, Balestrazzi A, Baiocchi S, Traversi C, Caporossi A (2007) Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: in vivo confocal microscopic evaluation. Clin Exp Ophthalmol 35(6):580–582
Mazzotta C, Traversi C, Baiocchi S, Caporossi O, Bovone C, Sparano M, Balestrazzi A, Caporossi A (2008) Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy in vivo: early and late modifications. Am J Ophthalmol 146(4):527–533
Mazzotta C, Caporossi T, Denaro R, Bovone C, Sparano C, Paradiso A, Baiocchi S, Caporossi A (2012) Morphological and functional correlations in riboflavin UV A corneal collagen cross-linking for keratoconus. Acta Ophthalmol 90(3):259–265
Mazzotta C, Traversi C, Caragiuli S, Rechichi M (2014) Pulsed vs continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT. Eye (Lond) 28(10):1179–1183
Meek K, Hayes S (2013) Corneal cross-linking--a review. Ophthalmic Physiol Opt 33(2):78–93
Mencucci R, Marini M, Paladini I, Sarchielli E, Sgambati E, Menchini U, Vannelli G (2010) Effects of riboflavin/UVA corneal cross-linking on keratocytes and collagen fibres in human cornea. Clin Exp Ophthalmol 38(1):49–56
Moshirfar M, Edmonds J, Behunin N, Christiansen S (2014) Current options in the management of pellucid marginal degeneration. J Refract Surg 30(7):474–485
Munnerlyn CR, Koons SJ, Marshall J (1988) Photorefractive keratectomy: a technique for laser refractive surgery. J Cataract Refract Surg 14(1):46–52
Nakayasu K, Tanaka M, Konomi H, Hayashi T (1986) Distribution of types I, II, III, IV and V collagen in normal and keratoconus corneas. Ophthalmic Res 18(1):1–10
Nishida T, Saika S (2011) Corneal and sclera: anatomy and physiology. In: Krachmer J, Mannis M, Holland E (eds) Cornea, vol 1. Mosby, Philadelphia, pp 4–24
O'Brart D, Chan E, Samaras K, Patel P, Shah S (2011) A randomised, prospective study to investigate the efficacy of riboflavin/ultraviolet A (370 nm) corneal collagen cross-linkage to halt the progression of keratoconus. Br J Ophthalmol 95(11):1519–1524
O'Brart D, Kwong T, Patel P, McDonald R, O'Brart N (2013) Long-term follow-up of riboflavin/ultraviolet A (370 nm) corneal collagen cross-linking to halt the progression of keratoconus. Br J Ophthalmol 97(4):433–437

Padmanabhan P, Dave A (2013) Collagen cross-linking in thin corneas. Indian J Ophthalmol 61 (8):422–424
Paik D, Wen Q, Braunstein R, Airiani S, Trokel S (2009) Initial studies using aliphatic beta-nitro alcohols for therapeutic corneal cross-linking. Invest Ophthalmol Vis Sci 50(3):1098–1105
Paik D, Solomon M, Wen Q, Turro N, Trokel S (2010) Aliphatic beta-nitroalcohols for therapeutic corneoscleral cross-linking: chemical mechanisms and higher order nitroalcohols. Invest Ophthalmol Vis Sci 51(2):836–843
Pérez-Santonja J, Artola A, Javaloy J, Alió J, Abad J (2009) Microbial keratitis after corneal collagen crosslinking. J Cataract Refract Surg 35(6):1138–1140
Pollhammer M, Cursiefen C (2009) Bacterial keratitis early after corneal crosslinking with riboflavin and ultraviolet-A. J Cataract Refract Surg 35(3):588–589
Price M, Tenkman L, Schrier A, Fairchild K, Trokel S, Price F (2012) Photoactivated riboflavin treatment of infectious keratitis using collagen cross-linking technology. J Refract Surg 28 (10):706–713
Rabinowitz YS (1995) Videokeratographic indices to aid in screening for keratoconus. J Refract Surg 11(5):371–379
Raiskup F, Spoerl E (2013) Corneal crosslinking with riboflavin and ultraviolet A. I. Principles. Ocul Surf 11(2):65–74
Raiskup F, Hoyer A, Spoerl E (2009) Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. J Refract Surg 25(9)
Raiskup F, Pinelli R, Spoerl E (2012) Riboflavin osmolar modification for transepithelial corneal cross-linking. Curr Eye Res 37(3):234–238
Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat L (2008) Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. J Cataract Refract Surg 34(5):796–801
Rama P, Di Matteo F, Matuska S, Paganoni G, Spinelli A (2009) Acanthamoeba keratitis with perforation after corneal crosslinking and bandage contact lens use. J Cataract Refract Surg 35 (4):788–791
Randleman B (2006) Post-laser in-situ keratomileusis ectasia: current understanding and future directions. Curr Opin Ophthalmol 17(4):406–412
Richoz O, Hammer A, Tabibian D, Gatzioufas Z, Hafezi F (2013a) The biomechanical effect of corneal collagen cross-linking (CXL) with riboflavin and UV-A is oxygen dependent. Transl Vis Sci Technol 2(7):6
Richoz O, Mavrakanas N, Pajic B, Hafezi F (2013b) Corneal collagen cross-linking for ectasia after LASIK and photorefractive keratectomy: long-term results. Ophthalmology 120 (7):1354–1359
Richoz O, Tabibian D, Hammer A, Majo F, Nicolas M, Hafezi F (2014) The effect of standard and high-fluence corneal cross-linking (CXL) on cornea and limbus. Invest Ophthalmol Vis Sci 55 (9):5783–5787
Sady C, Khosrof S, Nagaraj R (1995) Advanced Maillard reaction and crosslinking of corneal collagen in diabetes. Biochem Biophys Res Commun 214(3):793–797
Said D, Elalfy M, Gatzioufas Z, El-Zakzouk E, Hassan M, Saif M, Zaki A, Dua H, Hafezi F (2014) Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. Ophthalmology 121(7):1377–1382
Samaras K, O'Brart D, Doutch J, Hayes S, Marshall J, Meek K (2009) Effect of epithelial retention and removal on riboflavin absorption in porcine corneas. J Refract Surg 25(9):771–775
Schumacher S, Oeftiger L, Mrochen M (2011) Equivalence of biomechanical changes induced by rapid and standard corneal cross-linking, using riboflavin and ultraviolet radiation. Invest Ophthalmol Vis Sci 52(12):9048–9052
Seiler T, Koufala K, Richter G (1998) Iatrogenic keratectasia after laser in situ keratomileusis. J Refract Surg 14(3):312–317
Sharma N, Maharana P, Singh G, Titiyal J (2010) Pseudomonas keratitis after collagen crosslinking for keratoconus: case report and review of literature. J Cataract Refract Surg 36 (3):517–520

Sharma A, Nottage JM, Mirchia K, Sharma R, Mohan K, Nirankari VS (2012) Persistent corneal edema after collagen cross-linking for keratoconus. Am J Ophthalmol 154(6)
Sharma N, Roy S, Maharana P, Sehra S, Sinha R, Tandon R, Titiyal J, Vajpayee R (2014) Outcomes of corneal collagen crosslinking in pseudophakic bullous keratopathy. Cornea 33 (3):243–246
Sherif AM (2014) Accelerated versus conventional corneal collagen cross-linking in the treatment of mild keratoconus: a comparative study. Clin Ophthalmol 8:1435–1440
Shetty R, Kaweri L, Nuijts R, Nagaraja H, Arora V, Kumar R (2014a) Profile of microbial keratitis after corneal collagen cross-linking. Biomed Res Int 2014:340509. doi:10.1155/2014/340509, Epub 2014 Sept 11
Shetty R, Nagaraja H, Jayadev C, Shivanna Y, Kugar T (2014b) Collagen crosslinking in the management of advanced non-resolving microbial keratitis. Br J Ophthalmol 98(8):1033–1035
Shoulders M, Raines R (2009) Collagen structure and stability. Annu Rev Biochem 78(1):929–958
Sorkin N, Varssano D (2014) Corneal collagen crosslinking: a systematic review. Ophthalmologica 232(1):10–27
Spadea L (2010) Corneal collagen cross-linking with riboflavin and UVA irradiation in pellucid marginal degeneration. J Refract Surg 26(5):375–377
Spoerl E, Seiler T (1999) Techniques for stiffening the cornea. J Refract Surg 15(6):711–713
Spoerl E, Huhle M, Seiler T (1998) Induction of cross-links in corneal tissue. Exp Eye Res 66 (1):97–103
Spoerl E, Wollensak G, Dittert D-D, Seiler T (2004a) Thermomechanical behavior of collagen-cross-linked porcine cornea. Ophthalmologica 218(2):136–140
Spoerl E, Wollensak G, Seiler T (2004b) Increased resistance of crosslinked cornea against enzymatic digestion. Curr Eye Res 29(1):35–40
Stojanovic A, Chen X, Jin N, Zhang T, Stojanovic F, Raeder S, Utheim TP (2012) Safety and efficacy of epithelium-on corneal collagen cross-linking using a multifactorial approach to achieve proper stromal riboflavin saturation. J Ophthalmol 2012
Sugar J, Macsai M (2012) What causes keratoconus? Cornea 31(6):716–719
Sutton G, Lawless M, Hodge C (2014) Laser in situ keratomileusis in 2012: a review. Clin Exp Optom 97(1):18–29
Tao X, Yu H, Zhang Y, Li Z, Jhanji V, Ni S, Wang Y, Mu G (2013) Role of corneal epithelium in riboflavin/ultraviolet-A mediated corneal cross-linking treatment in rabbit eyes. Biomed Res Int 2013:624563. doi:10.1155/2013/624563, Epub 2013 Jun 27
Tomita M, Mita M, Huseynova T (2014) Accelerated versus conventional corneal collagen crosslinking. J Cataract Refract Surg 40(6):1013–1020
Touboul D, Efron N, Smadja D, Praud D, Malet F, Colin J (2012) Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedures for keratoconus. J Refract Surg 28(11):769–776
Tsatsos M, MacGregor C, Kopsachilis N, Anderson D (2014) Is accelerated corneal collagen cross-linking for keratoconus the way forward? Yes. Eye 28(7):784–785
Vajpayee R, Shafi S, Maharana P, Sharma N, Jhanji V (2015) Evaluation of corneal collagen cross-linking as an additional therapy in mycotic keratitis. Clin Exp Ophthalmol 43:103–107
van Dijk K, Parker J, Tong CM, Ham L, Lie JT, Groeneveld-van Beek EA, Melles GR (2014) Midstromal isolated Bowman layer graft for reduction of advanced keratoconus: a technique to postpone penetrating or deep anterior lamellar keratoplasty. JAMA Ophthalmol 132 (4):495–501
Vazirani J, Basu S (2013) Keratoconus: current perspectives. Clin Ophthalmol 7:2019–2030
Vazirani J, Vaddavalli P (2013) Cross-linking for microbial keratitis. Indian J Ophthalmol 61 (8):441–444
Vinciguerra R, Romano M, Camesasca F, Azzolini C, Trazza S, Morenghi E, Vinciguerra P (2013) Corneal cross-linking as a treatment for keratoconus. Ophthalmology 120(5):908–916

Wittig-Silva C, Chan E, Islam F, Wu T, Whiting M, Snibson G (2014) A randomized, controlled trial of corneal collagen cross-linking in progressive keratoconus: three-year results. Ophthalmology 121(4):812–821

Wollensak G (2010) Histological changes in human cornea after cross-linking with riboflavin and ultraviolet A. Acta Ophthalmol 88(2):e17–e18

Wollensak G, Iomdina E (2009a) Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. J Cataract Refract Surg 35(3):540–546

Wollensak G, Iomdina E (2009b) Long-term biomechanical properties of rabbit cornea after photodynamic collagen crosslinking. Acta Ophthalmol 87(1):48–51

Wollensak G, Spoerl E, Seiler T (2003a) Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 135(5):620–627

Wollensak G, Spoerl E, Seiler T (2003b) Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. J Cataract Refract Surg 29 (9):1780–1785

Wollensak G, Spoerl E, Wilsch M, Seiler T (2003c) Endothelial cell damage after riboflavin-ultraviolet-A treatment in the rabbit. J Cataract Refract Surg 29(9):1786–1790

Wollensak G, Spoerl E, Wilsch M, Seiler T (2004a) Keratocyte apoptosis after corneal collagen cross-linking using riboflavin/UVA treatment. Cornea 23(1):43–49

Wollensak G, Wilsch M, Spoerl E, Seiler T (2004b) Collagen fiber diameter in the rabbit cornea after collagen crosslinking by riboflavin/UVA. Cornea 23(5):503–507

Xia Y, Chai X, Zhou C, Ren Q (2011) Corneal nerve morphology and sensitivity changes after ultraviolet A/riboflavin treatment. Exp Eye Res 93(4):541–547

Yildirim A, Cakir H, Kara N, Uslu H, Gurler B, Ozgurhan EB, Colak HN (2014) Corneal collagen crosslinking for ectasia after laser in situ keratomileusis: long-term results. J Cataract Refract Surg 40(10):1591–1596

Yuksel N, Bilgihan K, Hondur A (2011) Herpetic keratitis after corneal collagen cross-linking with riboflavin and ultraviolet-A for progressive keratoconus. Int Ophthalmol 31(6):513–515

Zamora K, Males J (2009) Polymicrobial keratitis after a collagen cross-linking procedure with postoperative use of a contact lens: a case report. Cornea 28(4):474–476

Lens: Management of Cataract Surgery, Cataract Prevention, and Floppy Iris Syndrome

Joao Crispim and Wallace Chamon

Contents

J. Crispim
Department of Ophthalmology and Visual Sciences, Federal University of São Paulo (UNIFESP), Sao Paulo, Brazil

Illinois Eye and Ear Infirmary, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago (UIC), Chicago, IL, USA

W. Chamon (✉)
Department of Ophthalmology and Visual Sciences, Federal University of São Paulo (UNIFESP), Sao Paulo, Brazil

Illinois Eye and Ear Infirmary, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago (UIC), Chicago, IL, USA

Foton Oftalmologia, R. Olimpiadas, 134/51, São Paulo, SP 04551-000, Brazil
e-mail: wchamon@pobox.com

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_90

Abstract

According to the World Health Organization, cataract is the major cause of reversible visual impairment in the world. It is present as the cause of decreased visual acuity in 33% of the visual impaired citizens. With the increase of life expectancy in the last decades, the number of patients with cataract is expected to grow for the next 20 years. Nowadays, the only effective treatment for cataracts is surgery and its surgical outcomes have been increasingly satisfactory with the technological advancement.

Pharmaceutical development has been also responsible for surgical outcomes enhancement. This includes the development of new ophthalmic viscoelastic devices (OVDs), intraocular dyes, mydriatics, miotics, anesthetics, irrigating solutions, and antibiotics. However, the increased costs and demand for cataract surgery may be hard to meet in the future unless clinical preventive and curative options are evaluated.

In this chapter, we review the studies that addressed pharmacological applications in cataract.

Keywords

Anesthesia • Cataract • Cataract/Prevention • Mydriatics • Pharmacology • Viscoelastics

1 Management of Cataract Surgery

Phacoemulsification with a small ultrasonic tip was introduced by Kelman in 1967 and became the most common modality of cataract surgery (Kelman 1967). However, the learning curve of cataract surgery by means of phacoemulsification may increase intraoperative complications. To minimize complications, special surgical techniques had been developed for each surgical step. Cataract surgeons must be used to advances in technology to improve safe and constant outcomes. Intraocular manipulation during phacoemulsification generates turbulence of fluid and lens fragments that can cause endothelial cell damage and direct trauma to ocular structures (Dick et al. 1996). Knowledge of dyes used to enhance visualization of the capsular bag and proper use of ophthalmic viscoelastic devices (OVDs) allow for controlled surgical steps as well as for enhanced protection, reducing the prevalence of complications (Goldman and Karp 2007). The importance of adequate irrigating solutions during cataract surgery has been demonstrated. Solutions with ionic composition, pH, and osmolarity similar to aqueous humor have been associated with better outcomes (Lucena et al. 2011). The use of miotics at the end of the surgery may prevent intraocular lens (IOL) dislocation and the development of peripheral anterior synechiae (PAS) (Dieleman et al. 2012). The use of antibiotics has been associated with the decrease in pathologic microorganisms at the surgical site; nevertheless, multicenter trials have demonstrated that postoperative endophthalmitis is a complex multifactorial problem with different risk factors,

such as characteristics of the patient and the doctors, inadequate use of antibiotics, intraoperative complications as well as IOL and materials used. Introduction of perioperative intracameral antibiotics in cataract surgery could reduce the number of endophthalmitis infections (ESCRS Endophthalmitis Study Group 2007).

1.1 Mydriatics

Most of the intraocular procedures, especially cataract surgery, need sustained pupil dilation during the surgery. Although it is normally obtained with topical instillation of eyedrops before the surgery, a combination of topical and intracameral agents has also been used for mydriasis (Duffin et al. 1983; Corbett and Richards 1994; Liou and Chen 2001; Lundberg and Behndig 2007).

Intracameral mydriatics (ICM) without preoperative topical dilating drops has been used since 2003 with the use of a mixture containing a preservative-free solution of cyclopentolate 0.1%, phenylephrine 1.5%, and lidocaine 1% (Lundberg and Behndig 2008). Cyclopentolate has later been removed from this formulation because it did not add any additional mydriatic effect (Lundberg and Behndig 2008). It has also been recognized that adding of epinephrine continuously to the irrigating solution is not required when initial ICM is used (Lundberg and Behndig 2007). A different approach using a single dose of intracameral lidocaine 1% and continuous use of epinephrine in the irrigation solution has also conveyed acceptable dilatation (Cionni et al. 2003). Good mydriatic effect has been obtained with the use of a mixture of intracameral epinephrine 0.025% and lidocaine 0.75% (epi-S) associated with a preoperative drop of tropicamide 1% (Myers and Shugar 2009). The use of ICM has been proposed to improve the operating conditions in the intraoperative floppy iris syndrome by alleviating the iris movement during operation (Masket and Belani 2007; Chen et al. 2010).

The safety of ICM has been studied in animals (Liou et al. 2002; Kim et al. 2010) and humans (Bozkurt et al. 2010; Cakmak et al. 2010). Special attention has been paid to circulatory consequences during the use intracameral epinephrine. It was established that a significant decrease in heart frequency happened in patients who used topical mydriatics but not in those who used ICM. Because pulse deceleration is described with phenylephrine, the intracameral method may also have compensations in reducing the systemic side effects, especially in vulnerable persons (McKnight et al. 1995; Hempel et al. 1999).

In summary, ICM is an option to modern cataract surgery and delivers sufficient pupil dilatation, without producing quantifiable ocular side effects or negatively influencing the phacoemulsification procedure when compared to topical mydriatics (Behndig and Eriksson 2004; Johansson et al. 2007).

1.2 Anesthetics

Cataract surgery anesthesia evolved from general to topical anesthesia (TA) passing through a long period of regional anesthesia (RA). Instillation of topical anesthetic eyedrops delivers adequate corneal numbness, propitiating cataract surgery by phacoemulsification when akinesia is not mandatory (Schutz and Mavrakanas 2010). Topical anesthesia has progressively been used for cataract surgery worldwide, accounting for up to 60% of procedures (Wagle et al. 2007) and is the method of choice for patients receiving anticoagulant or antiplatelet treatment (Barequet et al. 2007).

Although some surgeons exclusively perform TA for routine phacoemulsification, the efficacy of TA may be limited due to incomplete analgesia. In one study, 71% of patients who had both eyes operated and received retrobulbar anesthesia (RBA) in one eye and TA in the contralateral eye preferred RBA (Boezaart et al. 2000). Intraoperative safety is more frequently achieved with RBA (Rebolleda et al. 2001; Gombos et al. 2007) or sub-Tenon's injection (STI) (Sekundo et al. 2004) than with TA.

Although TA is simpler to achieve and evades the potential threats of needle techniques, its safety has been only confirmed through a reduction of sight-threatening complication frequency when compared to RBA (Eke and Thompson 2007), which may be explained by the very low rate of complications with RA. A theoretical drawback of TA is the consequence of the absence of akinesia in possible intraocular pressure fluctuation that may hypothetically make the surgery more dangerous (Davison et al. 2007). In a randomized, open-labeled, comparative study of unselected patients, it has been observed a desirable decrease in rate of vitreous loss (0.4% vs 2.5%) and an undesirable increase in iris prolapse (1.7% vs 0.4%) in the TA group, possibly reflecting eye hypertonia due to the lack of akinesia (Jacobi et al. 2000). A low degree of surgical complications of cataract surgery performed under TA has been shown in a large cases series (Shaw et al. 2007). However, a more recent meta-analysis review has demonstrated that, when compared with STI, TA is related with a twofold increase in posterior capsule rupture, demanding anterior vitrectomy (Davison et al. 2007). Likewise, TA was recognized as a risk factor for dislocation of nuclear fragments into the vitreous (Mahmood et al. 2008). Hence, TA should be restricted to intended easy procedures made by experienced surgeons in selected patients. For manual extracapsular cataract extraction (ECCE), akinesia may still be obligatory and TA is doubtful. This may be the case when phacoemulsification is not accessible for technical or economical reasons (Waddell et al. 2004; Bourne et al. 2004).

Improvement in TA efficacy has been explored, such as the use of anesthetics with long-acting effect, for example, levobupivacaine or ropivacaine (Borazan et al. 2008). Intracameral injection of a small volume (0.1 mL) of local anesthetic at the beginning of surgery has been suggested to improve analgesia (Karp et al. 2001). Although the corneal endothelium safety of this method has been established (Heuerman et al. 2002), no significant analgesic advantage over simple TA has been demonstrated by appropriately designed trials (Ezra and Allan 2007). The lack of additional analgesia

may be explained by the fact that it is not associated with the intracameral concentration of the drug (Bardocci et al. 2003). The effectiveness of sponges soaked with local anesthetic placed into the conjunctival fornices and soluble local anesthetic implants require additional documentation (Mahe et al. 2005). Instilling lidocaine jelly instead of eyedrops seems to improve the quality of analgesia of the anterior segment (Barequet et al. 1999). Nevertheless, lidocaine jelly has been related with an increase in postoperative incidence of endophthalmitis, as explained by the French sanitary agency in 2004 (AFSSAPS 2004). The most plausible reason for this result is that if the jelly was applied first on the eye, it would have acted as a barrier, thus resulting in inadequate eye disinfection. Therefore, the problem is possibly the mistaken sequence of application rather than the jelly itself. As a matter of fact, TA has been related with a 3.8-fold increase in endophthalmitis frequency compared with RBA (Garcia-Arumi et al. 2007). In order to improve the quality of analgesia and avoid toxicity, specifically formulated topical lidocaine or tetracaine jellies should replace jellies designed for use in urology and commonly used in ophthalmology (Amiel and Koch 2007).

1.3 Dyes

The efficacious achievement of a continuous curvilinear capsulorhexis (CCC) is a critical step in performing phacoemulsification that can be improved by staining. Capsular staining will be especially useful in eyes presenting reduced intraoperative red reflex, pediatric cataract surgery, and for surgeons in training with new intraoperative techniques demanding superb visualization of the anterior capsule (Goldman and Karp 2007).

Literature has evaluated different dyes, such as trypan blue, indocyanine green (ICG), crystal violet, fluorescein, Brilliant Blue G (BBG), and gentian violet. Although ICG and trypan blue have been used widely in humans without important anterior-segment toxicity under normal situations, Food and Drug Administration (FDA) accepts only trypan blue for use in cataract surgery. The use of ICG and fluorescein staining involves an off-label use of an FDA-approved product. A new dye (BBG) was tested in vitro and demonstrated that it was effective in staining the anterior capsule at very low concentration (0.25 mg/mL) and revealed no apparent toxicity at concentrations 40 times as high as by using microscopic imaging techniques. Both transmission and scanning electron microscopy indicated endothelial injury in the ICG and trypan blue groups; however, the endothelial cells in the BBG group were unchanged (Hisatomi et al. 2006).

The Ophthalmic Technology Assessment Committee Anterior Segment Panel of the American Academy of Ophthalmology reported, in 2006, an analysis of the literature on capsular staining for cataract surgery and established level III evidence (case series and case reports) that ICG and trypan blue were both easier to use and visualize than fluorescein (Jacobs et al. 2006). The committee reported that there is evidence of the safety of trypan blue in the anterior chamber; however, limited data is available to demonstrate the safety of ICG.

Several approaches have been used for the application of the capsular dye, such as, application under an OVD (Melles et al. 1999; Caporossi et al. 2005), a mix of OVD and dye (Dada et al. 2002), and by OVD coupled with balanced salt solution (Khokhar et al. 2003; Marques et al. 2004). Though, the most common method includes instillation of a small air bubble into the anterior chamber with a 27-gauge cannula and insertion of the dye underneath the air bubble (Ozturk and Osher 2006).

While capsular staining starts to be more commonly used, it is essential to stay aware against inconvenient administration of a toxic dye. Authors have described cases indicating the severe toxic outcome of intraocular methylene blue 1% (Brouzas et al. 2006; Timucin et al. 2016). In both reports, trypan blue was requested; however, methylene blue was accidentally taken and injected. This mistake led to iatrogenic corneal decompensation.

In conclusion, capsular stain with trypan blue is harmless, FDA-approved, and aids as a valuable adjunct to facilitate greater visualization of the anterior capsule. The critical step of CCC can now be securely achieved in most clinical conditions.

1.4 Viscoelastics

Considering that corneal endothelium has minimal regenerative ability (American Academy of Ophthalmology (AAO) 1997), any injury to the endothelial cell layer results in a decreased cell density and affects the ability of the endothelium to maintain hydrostatic balance and resist further insult. Endothelial cell density (ECD) needed to prevent corneal swelling reaches a critical threshold at less than 500–1,000 cells/mm^2 (Cheng et al. 1981). Bullous keratopathy is a serious complication, consequence of decrease in ECD, that may occur in up to 0.3% of patients following cataract surgery (Powe et al. 1994). Due to the acceleration in cell loss rate after surgery, pseudophakic bullous keratopathy may occur many years after cataract surgery and is the third most common cause of penetrating keratoplasty (Fasolo et al. 2006). Long-term endothelial cell loss is influenced by the amount of surgical trauma (Ravalico et al. 2003) and the mean rate of endothelial cell loss is significantly higher than the physiological rate even 2 years after surgery, amounting to 0.9% per year (Lesiewska-Junk et al. 2002). Careful microsurgery with the use of protective OVD is mandatory to minimize endothelial cell loss (Coster 2001).

Different OVDs are available nowadays and may be classified according to their two main properties: viscosity and cohesion (Arshinoff and Jafari 2005). Zero-shear viscosity is related to space creation and maintenance in surgery, whereas cohesion (the opposite of dispersion) is related to the coating properties and retention capability of the OVD during surgery. Highly dispersive OVDs are more difficult to aspirate at the end of the procedure (Arshinoff and Jafari 2005). It's well known that hypermature cataracts require higher ultrasonic energy that contributes to undesirable OVD intraoperative washout. Dispersive OVDs, when properly utilized, are able maintain their coating characteristics for longer periods (Storr-Paulsen et al. 2007). In hard lens nucleus, the "soft-shell technique," which combines cohesive

OVD under a shell of dispersive OVD, is considered safe and effective in protecting corneal endothelial cells during cataract surgery (Miyata et al. 2002).

1.5 Irrigating Solutions

Different solutions have been used in order to minimize chemical endothelium cell damage during intraocular surgery. The designation of balanced salt solution (BSS®) has been used for more physiological solutions with controlled pH, osmolarity, and ionic composition (Merrill et al. 1960). Later, the addition of glutathione (GSH), glucose, and bicarbonate to the irrigation solution (BSS Plus®) demonstrated better endothelial cell function and survival in vitro (Edelhauser et al. 1978). Studies have shown that enhanced balanced salt solutions such as BSS Plus® offer features similar to those of the aqueous humor to preserve constant intraocular circumstances (Araie 1986).

Dextrose bicarbonate Lactated Ringer's solution for irrigation has been described to be as efficient as enriched BSS® for nontraumatic cataract surgery by phacoemulsification and extracapsular (Puckett et al. 1995; Kiss et al. 2003). However, conflicting studies have demonstrated superiority of BSS® and BSS Plus® over Ringer's solution on the first postoperative day (Joussen et al. 2000; Vasavada et al. 2009).

Theoretically, the presence of magnesium, sodium phosphate, sodium bicarbonate, dextrose, and GSH in BSS Plus® would improve the Mg-ATPase endothelial pump and maintain the intercellular junctions (Edelhauser 2000). In addition, aqueous humor, BSS Plus®, and Lactated Ringer's present pH of 7.38, 7.40, and 6.4 and osmolarities of 304, 305, and 260 mOsm, respectively. Therefore, Lactated Ringer's solution is hypotonic and slightly acidic when compared to BSS Plus® and aqueous humor (Nuyts et al. 1995).

In vivo studies comparing different irrigating solutions have shown that postoperative central corneal thickness (CCT) and ECD are not influenced by irrigation volume and time of the surgery; however, they are dependent on the solution's specification (Matsuda et al. 1991). Cornea thickness increases immediately after surgery, when the pump and the barrier functions of the endothelium are deteriorated, and is a measurement of the surgically induced endothelial damage but, in a long-term basis, ECD is not associated with CCT (Lucena et al. 2011). After a couple of months of the surgery, ECD remains irreversibly changed; however, cell function is frequently reestablished, and there is a decrease in CCT to preoperative values (Kiss et al. 2003).

In conclusion, for uncomplicated and, especially, for surgeries that may need a higher volume of irrigation solution or longer phacoemulsification time, Ringer's solution causes a higher corneal endothelial cell decrease when compared to BSS Plus®.

1.6 Miotics

The use of miotics, such as carbachol or physostigmine, causes intense pupil reduction. This pupil effect of miotics is believed to prevent dislocation of sulcus-placed IOLs, and development of peripheral anterior synequiae (PAS) by pulling the iris away from the anterior chamber angle and incisions (Dieleman et al. 2012). Miotics have also been preconized to prevent increase in intraocular pressure after surgery (Phillipall et al. 1997).

The use of miotics has been reduced, because many ophthalmologists no longer believe it to be of any advantage and because of undesirable effects, such as muscular twitches, brow ache, and decreased visual acuity caused by diffraction. The usefulness of miotics after phacoemulsification in preventing PAS, lens dislocation, and high postoperative intraocular pressure in patients with normal eyes has lost its rationale and, therefore, should not be recommended as routine use after phacoemulsification (Dieleman et al. 2012; Linden and Alm 1997).

1.7 Prophylactic Antibiotics

The use of prophylactic antibiotics is still controversial to the current practice. In 2007, the European Society of Cataract & Refractive Surgeons (ESCRS) conducted a multicentric study throughout Europe to compare intracameral cefuroxime injected at the end of surgery and topical levofloxacin given immediately preoperatively (within 1 h of surgery) and up to 15 min following surgery in 16,603 participants. One group received only intracameral cefuroxime, one group received only topical levofloxacin, one group received both intracameral cefuroxime and topical levofloxacin, and one group received neither intervention. Povidone iodine was given for antisepsis at the time of procedure and topical levofloxacin was given to all eyes starting the morning after surgery (ESCRS 2007). Its design permitted the assessment of both topical and intracameral antibiotics and comprised a sample size appropriate to yield statistically significant results, although a high incidence of endophthalmitis in the control group could have overestimated the effect of intracameral antibiotics. Authors recommended that intracameral antibiotic injection is beneficial in reducing the risk of post-cataract surgery endophthalmitis.

Since 2007, the use of intracameral cefuroxime has varied broadly. In the UK, approximately 50% of contributors report the use of intracameral antibiotic (Gore et al. 2009), while in the USA, where the approach has not been approved by FDA, acceptance has been more limited. Results of the 2011 American Society of Cataract and Refractive Surgery (ASCRS) member survey revealed fewer than 20% of surgeons using intracameral antibiotics, and preferentially among the high volume surgeons (Vazirani and Basu 2013; Chang et al. 2008; Keay et al. 2012). Surgeons not using intracameral antibiotics mention concerns regarding dilution errors and risk of contamination when compounding the drugs for doses required for ocular injection (Gore et al. 2009).

2 Cataract Prevention

Medical delaying, prevention, or treatment of cataracts has been tried for many decades. Although most of the drugs have addressed antioxidant effects, poor penetration of pharmacological agents into the lens has proved to be a major obstacle for successful treatments. Although, to this moment, no proven effective drug has been developed, and many failed to prove their effectiveness, some are commercially available in different countries (Harding 1991).

2.1 Sorbitol Lowering Agents

Although, high aldose reductase activity and increased sorbitol levels have been associated with cataracts in rodents, sorbitol is unlikely to accumulate in human lens, due to specific high activity of sorbitol lowering enzymes in humans (Harding 1991; Abdelkader et al. 2011).

2.2 Pirenoxine

Pirenoxine is a drug capable of binding to molecules associated with cataract formation in animals: Ca^{2+} and selenite. Although it has never been proved effective, pirenoxine is commercially available in many countries such as Brazil, China, Japan, and others (Harding 1991; Maclean and Taylor 1981; Testa et al. 1987).

2.3 Nonsteroidal Anti-inflammatory Drugs

Although no definite action mechanism has been proposed, aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), such as paracetamol and ibuprofen, have been retrospectively associated with lower prevalence of cataract (Harding 1991) and have been shown to prevent cataract in animals (Gupta et al. 1984; Swamy and Abraham 1989). Nowadays the evidence of NSAIDs effect in preventing cataract in humans is inconsistent (van Heyningen and Harding 1986; Harding and van Heyningen 1988; Klein et al. 2001; Chew et al. 1992; Hankinson et al. 1993). Recent evaluation of Age-Related Eye Disease Study (AREDS) data did not demonstrate a protective effect of NSAID intake (Chang et al. 2011). The influence of NSAID should be further evaluated, but studies have demonstrated that a dose as high as 15,000 md/day may be needed to achieve a protective effect in humans (Swamy and Abraham 1989).

2.4 Protein Stabilizers

It has been demonstrated that systemic and topical use of Bendazac, its metabolite 5-hydroxyl bendazac, its water-soluble lysine salt and analogues protects lens proteins from aggregation under different models (Lewis et al. 1986; Lewis and Harding 1988; Balfour and Clissold 1990; Ahuja et al. 2008; Shen et al. 2010). Bendazac has been effective in preventing cataract in small samples clinical trials for short period of time (Bron et al. 1987; Rhee et al. 1987). Bendazac lysine eyedrops is available in Argentina, South Korea, and many European countries.

2.5 Antioxidants

Antioxidants or reducing agents inhibit oxidation by accepting at least two electrons from reactive oxygen species (free radicals) and play a major role in maintaining lens protein structure (Umapathy et al. 2013). Many molecules had their anticataractogenic effect studied, such as: GSH, its precursors cysteine and cysteine prodrug L-2-oxothiazolidine-4-carboxylic acid (OTZ) as well as N-acetyl carnosine, N-acetylcysteine, N-acetylcysteine amide, diosmin, curcumin, quercetin, β-carotene, lutein, and lycopene (Abdelkader et al. 2015; Manikandan et al. 2009). The great challenge for the therapeutic use of antioxidants is to achieve high levels of bioavailable molecules in the anterior chamber, which seems to be impossible through the circulatory system (Umapathy et al. 2013). Water insoluble molecules such as natural flavonoids (diosmin, curcumin, and quercetin) and carotenoids (β-carotene, lutein, and lycopene) are known by their poor bioavailability, which could weaken their potential for topical use (Freag et al. 2013).

Anticataractogenic effect of topical N-acetyl carnosine (1% and 2%) has been demonstrated in humans and animals (Williams and Muunday 2006; Babizhayev et al. 2002, 2009), but larger clinical trials are needed in order to evaluate its potential benefits.

Potential inhibition of oxidative stress and cataract development by caffeine in mice with selenite-induced cataracts has been demonstrated (Varma et al. 2010). Although there are no clinical trials in humans that studied the relationship between caffeine intake and risk of cataract, recent observation indicated that caffeine could have a general anticataractogenic effect when used pharmacologically (Wegener and Laser-Junga 2009).

A large randomized, double-masked, placebo-controlled clinical trial failed to demonstrate prophylactic effect of vitamins E and C on age-related cataract (Christen et al. 2010). AREDS did not demonstrate any influence of antioxidant formulation on the progression of age-related lens opacities (AREDS 2001).

2.6 Lanosterol

Lanosterol synthase is an enzyme that acts in the pathway of cholesterol synthesis, preventing intracellular protein aggregation of various cataract-causing mutant crystallins. It has been demonstrated that lanosterol lessened lens protein aggregation and improved lens clarity in dogs, cultured rabbit lenses, and cell culture. Authors also identified two families with hereditary congenital cataract that presented mutation in LSS gene (lanosterol encoding gene) located on chromosome 21 (Zhao et al. 2015).

References

Abdelkader H et al (2011) New therapeutic approaches in treatment of diabetic keratopathy. Clin Exp Opthalmol 39:259–270

Abdelkader H, Alany RG, Pierscionek B (2015) Age-related cataract and drug therapy: opportunities and challenges for topical antioxidant delivery to the lens. J Pharm Pharmacol 67:537–550

Agence Francaise pour la Securite Sanitaire et des Produits de Sante (AFSSAPS) (2004) Me susage de Xylocaine 2%, gel uretral en seringue preremplie: endophtalmies rapporte es lors du traitement chirurgical de la cataracte. Vigilances (2):2 [online]. http://www.afssaps.fr/var/afssaps_site/storage/original/application/9095a6c4be02687266bd4b042dad2229.pdf

Ahuja M, Dhake AS, Sharma SK, Majumdar DK (2008) Topical ocular delivery of NSAIDs. AAPS J 10(2):229–241

American Academy of Ophthalmology (AAO) (1997) Corneal endothelial photography: three-year revision. Ophthalmology 104:1360–1365

Amiel H, Koch PS (2007) Tetracaine hydrochloride 0.5% versus lidocaine 2% jelly as a topical anesthetic agent in cataract surgery: comparative clinical trial. J Cataract Refract Surg 33:98–100

Araie M (1986) Barrier function of corneal endothelium and the intraocular irrigating solutions. Arch Ophthalmol 104:435–438

AREDS (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. Arch Ophthalmol 119:1439–1452

Arshinoff SA, Jafari M (2005) New classification of ophthalmic viscosurgical devices. J Cataract Refract Surg 31:2167–2171

Babizhayev MA et al (2002) Efficacy of N-acetylcarnosine in the treatment of cataracts. Drugs R D 3:87–103

Babizhayev MA et al (2009) N-Acetylcarnosine sustained drug delivery eye drops to control the signs of ageless vision: glare sensitivity, cataract amelioration and quality of vision currently available treatment for the challenging 50,000-patient population. Clin Interv Aging 4:31–50

Balfour JA, Clissold SP (1990) Bendazac lysine. A review of its pharmacological properties and therapeutic potential in the management of cataracts. Drugs 39:575–596

Bardocci A, Lofoco G, Perdicaro S et al (2003) Lidocaine 2% gel versus lidocaine 4% unpreserved drops for topical anesthesia in cataract surgery: a randomized controlled trial. Ophthalmology 110:144–149

Barequet IS, Soriano ES, Green WR et al (1999) Provision of anesthesia with single application of lidocaine gel. J Cataract Refract Surg 25:626–631

Barequet IS, Sachs D, Priel A et al (2007) Phacoemulsification of cataract in patients receiving coumadin therapy: ocular and hematologic risk assessment. Am J Ophthalmol 144:719–772

Behndig A, Eriksson A (2004) Evaluation of surgical performance with intracameral mydriatics in phacoemulsification surgery. Acta Ophthalmol Scand 82:144–147

Boezaart A, Berry R, Nell M (2000) Topical anesthesia versus retrobulbar block for cataract surgery: the patient's perspective. J Clin Anesth 12:58–60
Borazan M, Karalezli A, Akova YA et al (2008) Comparative clinical trial of topical anaesthetic agents for cataract surgery with phacoemulsification: lidocaine 2% drops, levobupivacaine 0.75% drops, and ropivacaine 1% drops. Eye 22:425–429
Bourne RR, Minassian DC, Dart JK et al (2004) Effect of cataract surgery on the corneal endothelium: modern phacoemulsification compared with extracapsular surgery. Ophthalmology 11:679–685
Bozkurt E, Yazici AT, Pekel G, Albayrak S, Cakir M, Pekel E et al (2010) Effect of intracameral epinephrine use on macular thickness after uneventful phacoemulsification. J Cataract Refract Surg 36:1380–1384
Bron AJ, Brown NA, Sparrow JM, Shun-Shin GA (1987) Medical treatment of cataract. Eye 1:542–555
Brouzas D, Droutsas D, Charakidas A et al (2006) Severe toxic effect of methylene blue 1% on iris epithelium and corneal endothelium. Cornea 25:470–471
Cakmak HB, Cagil N, Dal D, Simavli H, Arifoglu HB, Simsek S (2010) Effects of intracameral use of adrenalin solution with preservative on corneal endothelium. Cutan Ocul Toxicol 29:41–49
Caporossi A, Balestrazzi A, Alegente M et al (2005) Trypan blue staining of the anterior capsule: the one-drop technique. Ophthalmic Surg Lasers Imaging 36:432–434
Chang MA, Congdon NG, Baker SK, Bloem MW, Savage H, Sommer A (2008) The surgical management of cataract: barriers, best practices and outcomes. Int Ophthalmol 28(4):247–260
Chang JR, Koo E, Agron E et al (2011) Risk factors associated with incident cataracts and cataract surgery in the Age-Related Eye Disease Study (AREDS): AREDS report number 32. Ophthalmology 118:2113–2119
Chen AA, Kelly JP, Bhandari A, Wu MC (2010) Pharmacologic prophylaxis and risk factors for intraoperative floppy-iris syndrome in phacoemulsification performed by resident physicians. J Cataract Refract Surg 36:898–905
Cheng H, Law AB, McPherson K et al (1981) Longitudinal study of intraocular lens implants after intracapsular cataract extraction. Complete follow-up of the first 7 years. Trans Ophthalmol Soc U K 101:79–83
Chew EY, Williams GA, Burton TC, Barton FB, Remaley NA, Ferris FL III (1992) Aspirin effects on the development of cataracts in patients with diabetes mellitus. Early treatment diabetic retinopathy study report 16. Arch Ophthalmol 110:339–342
Christen WG, Glynn RJ, Sesso HD et al (2010) Age-related cataract in a randomized trial of vitamins E and C in men. Arch Ophthalmol 128:1397–1405
Cionni RJ, Barros MG, Kaufman AH, Osher RH (2003) Cataract surgery without preoperative eyedrops. J Cataract Refract Surg 29:2281–2283
Corbett MC, Richards AB (1994) Intraocular adrenaline maintains mydriasis during cataract surgery. Br J Ophthalmol 78:95–98
Coster DJ (2001) Corneal trauma. Cornea. BMJ Publishing, London, p 117
Dada VK, Sudan R, Sharma N, Dada T (2002) Trypan blue with viscoelastic agent. J Cataract Refract Surg 28:205–206
Davison M, Padroni S, Bunce C et al (2007) Sub-Tenon's anaesthesia versus topical anaesthesia for cataract surgery. Cochrane Database Syst Rev 18(3):CD006291
Dick HB, Kohnen T, Jacobi FK et al (1996) Long-term endothelial cell loss following phacoemulsification through a temporal clear corneal incision. J Cataract Refract Surg 22:63–71
Dieleman M, Wubbels RJ, De Waard PW (2012) Miotics after modern cataract surgery are history. J Ocul Pharmacol Ther 28:98–101
Duffin RM, Pettit TH, Straatsma BR (1983) Maintenance of mydriasis with epinephrine during cataract surgery. Ophthalmic Surg 14:41–45
Edelhauser HF (2000) The resiliency of the corneal endothelium to refractive and intraocular surgery. Cornea 19:263–273

Edelhauser HF, Gonnering R, Van Horn DL (1978) Intraocular irrigating solutions. A comparative study of BSS Plus and lactated Ringer's solution. Arch Ophthalmol 96:516–520

Eke T, Thompson JR (2007) Serious complications of local anaesthesia for cataract surgery: a 1 year national survey in the United Kingdom. Br J Ophthalmol 91:470–475

Endophthalmitis Study Group, European Society of Cataract & Refractive Surgeons (2007) Prophylaxis of postoperative endophthalmitis following cataract surgery: results of the ESCRS multicenter study and identification of risk factors. J Cataract Refract Surg 33:978–988

Ezra DG, Allan BD (2007) Topical anaesthesia alone versus topical anaesthesia with intracameral lidocaine for phacoemulsification. Cochrane Database Syst Rev 18(3):CD005276

Fasolo A, Frigo AC, Bohm E et al (2006) The CORTES study: corneal transplant indications and graft survival in an Italian cohort of patients. Cornea 25:507–515

Freag MS, Elnaggar YS, Abdallah OY (2013) Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: optimization and ex vivo permeation. Int J Nanomedicine 8:2385–2397

Garcia-Arumi J, Fonollosa A, Sararols L et al (2007) Topical anesthesia: possible risk factor for endophthalmitis after cataract extraction. J Cataract Refract Surg 33:989–992

Goldman JM, Karp CL (2007) Adjunct devices for managing challenging cases in cataract surgery: capsular staining and ophthalmic viscosurgical devices. Curr Opin Ophthalmol 18:52–57

Gombos K, Jakubovits E, Kolos A et al (2007) Cataract surgery anaesthesia: is topical anaesthesia really better than retrobulbar? Acta Ophthalmol Scand 85:309–316

Gore DM, Angunawela RI, Little BC (2009) United Kingdom survey of antibiotic prophylaxis practice after publication of the ESCRS Endophthalmitis Study. J Cataract Refract Surg 35 (4):770–773

Gupta PP, Pandey DN, Pandey DJ, Sharma AL, Srivastava RK, Mishra SS (1984) Aspirin in experimental cataractogenesis. Indian J Med Res 80:703–707

Hankinson SE, Seddon JM, Colditz GA, Stampfer MJ, Rosner B, Speizer FE, Willett WC (1993) A prospective study of aspirin use and cataract extraction in women. Arch Ophthalmol 111:503–508

Harding J (1991) Prevention and therapy. In: Harding J (ed) Cataract: biochemistry, epidemiology and pharmacology. Chapman and Hall, London, pp 218–249

Harding JJ, van Heyningen R (1988) Drugs, including alcohol, that act as risk factors for cataract, and possible protection against cataract by aspirin-like analgesics and cyclopenthiazide. Br J Ophthalmol 72:809–814

Hempel S, Senn P, Pakdaman F, Schmid MK, Suppiger M, Schipper I (1999) Perioperative circulatory side effects of topical 5% phenylephrine for mydriasis. Klin Monbl Augenheilkd 215:298–304

Heuerman T, Hartman C, Anders N (2002) Long term endothelial cell loss after phacoemulsification: peribulbar anesthesia versus intracameral lidocaine 1%: prospective randomized study. J Cataract Refract Surg 28:638–643

Hisatomi T, Enaida H, Matsumoto H et al (2006) Staining ability and biocompatibility of brilliant blue G: preclinical study of brilliant blue G as an adjunct for capsular staining. Arch Ophthalmol 124:514–519

Jacobi PC, Dietlein TS, Jacobi FK (2000) A comparative study of topical vs retrobulbar anesthesia in complicated cataract surgery. Arch Ophthalmol 118:1037–1043

Jacobs DS, Cox TA, Wagoner MD, American Academy of Ophthalmology, Ophthalmic Technology Assessment Committee Anterior Segment Panel et al (2006) Capsule staining as an adjunct to cataract surgery: a report from the American Academy of Ophthalmology. Ophthalmology 113:707–713

Johansson M, Lundberg B, Behndig A (2007) Optical coherence tomography evaluation of macular edema after phacoemulsification surgery with intracameral mydriatics. J Cataract Refract Surg 33:1436–1441

Joussen AM, Barth U, Cubuk H et al (2000) Effect of irrigating solution and irrigation temperature on the cornea and pupil during phacoemulsification. J Cataract Refract Surg 26:392–397

Karp CL, Cox TA, Wagoner MD et al (2001) Intracameral anesthesia: a report by the American Academy of Ophthalmology. Ophthalmology 108:1704–1710
Keay L, Gower EW, Cassard SD, Tielsch JM, Schein OD (2012) Postcataract surgery endophthalmitis in the United States: analysis of the complete 2003 to 2004 Medicare database of cataract surgeries. Ophthalmology 119(5):914–922
Kelman CD (1967) Phaco-emulsification and aspiration. A new technique of cataract removal. A preliminary report. Am J Ophthalmol 64:23–35
Khokhar S, Pangtey MS, Panda A, Sethi HS (2003) Painting technique for staining the anterior lens capsule. J Cataract Refract Surg 29:435–436
Kim EC, Park SH, Kim MS (2010) A comparison of pupil dilation and induction of corneal endothelial apoptosis by intracameral 1% lidocaine versus 1:100,000 epinephrine in rabbits. J Ocul Pharmacol Ther 26(6):563–570. doi:10.1089/jop.2010.0078
Kiss B, Findl O, Menapace R et al (2003) Corneal endothelial cell protection with a dispersive viscoelastic material and a irrigating solution during phacoemulsification. Low-cost versus expensive combination. J Cataract Refract Surg 29:733–740
Klein BE, Klein R, Lee KE, Danforth LG (2001) Drug use and five-year incidence of age-related cataracts: The Beaver Dam Eye Study. Ophthalmology 108:1670–1674
Lesiewska-Junk H, Kaluzny J, Malukiewicz-Wisniewska G (2002) Long-term evaluation of endothelial cell loss after phacoemulsification. Eur J Ophthalmol 12:30–33
Lewis BS, Harding J (1988) The major metabolite of bendazac inhibits the glycosylation of soluble proteins: a possible mechanism for a delay in cataractogenesis. Exp Eye Res 47:217–225
Lewis BS, Rixon KC, Harding JJ (1986) Bendazac prevents cyanate binding to soluble lens proteins and cyanate-induced phase separation opacities in vitro: a possible mechanism by which bendazac could delay cataract. Exp Eye Res 43:973–979
Linden C, Alm A (1997) Latanoprost and physostigmine have mostly additive ocular hypotensive effects in human eyes. Arch Ophthalmol 115:857–861
Liou SW, Chen CC (2001) Maintenance of mydriasis with one bolus of epinephrine injection during phacoemulsification. J Ocul Pharmacol Ther 17:249–253
Liou SW, Chiu CJ, Wang IJ (2002) Effects of intraocular epinephrine on the corneal endothelium of rabbits. J Ocul Pharmacol Ther 18:469–473
Lucena DR, Ribeiro MS, Messias A, Bicas HE, Scott IU, Jorge R (2011) Comparison of corneal changes after phacoemulsification using BSS Plus versus lactated Ringer's irrigating solution: a prospective randomised trial. Br J Ophthalmol 95:485–489
Lundberg B, Behndig A (2007) Intracameral mydriatics in phacoemulsification surgery obviate the need for epinephrine irrigation. Acta Ophthalmol Scand 85:546–550
Lundberg B, Behndig A (2008) Separate and additive mydriatic effects of lidocaine hydrochloride, phenylephrine, and cyclopentolate after intracameral injection. J Cataract Refract Surg 34:280–283
Maclean H, Taylor CJ (1981) An objective staging for cortical cataract in vivo aided by pattern analysing computer. Exp Eye Res 33:597–602
Mahe I, Mouly S, Jarrin I et al (2005) Efficacy and safety of three ophthalmic inserts for topical anaesthesia of the cornea: an exploratory comparative dose-ranging, double-blind, randomized trial in healthy volunteers. Br J Clin Pharmacol 59:220–226
Mahmood S, von Lany H, Cole MD et al (2008) Displacement of nuclear fragments into the vitreous complicating phacoemulsification surgery in the UK: incidence and risk factors. Br J Ophthalmol 92:488–492
Manikandan R, Thiagarajan R, Beulaja S, Chindhu S, Mariammal K, Sudhandiran G, Arumugam M (2009) Anticataractogenic effect of curcumin and aminoguanidine against selenium-induced oxidative stress in the eye lens of Wistar rat pups: an in vitro study using isolated lens. Chem Biol Interact 181:202–209
Marques DM, Marques FF, Osher RH (2004) Three-step technique for staining the anterior lens capsule with indocyanine green or trypan blue. J Cataract Refract Surg 30:13–16

Masket S, Belani S (2007) Combined pre-operative topical atropine sulfate 1% and intracameral nonpreserved epinephrine hydrochloride 1:4000 for management of intraoperative floppy-iris syndrome. J Cataract Refract Surg 33:580–582

Matsuda M, Kinoshita S, Ohashi Y et al (1991) Comparison of the effects of intraocular irrigating solutions on the corneal endothelium in intraocular lens implantation. Br J Ophthalmol 75:476–479

McKnight JA, Rooney DP, Whitehead H, Atkinson AB (1995) Blood pressure responses to phenylephrine infusions in subjects with Cushing's syndrome. J Hum Hypertens 9:855–858

Melles GRJ, de Waard PWT, Pameyer JH, Beekhuis WH (1999) Trypan blue capsule staining to visualize the capsulorhexis in cataract surgery. J Cataract Refract Surg 25:7–9

Merrill DL, Fleming TC, Girard LJ (1960) The effects of physiologic balanced salt solutions and normal saline on intraocular and extraocular tissues. Am J Ophthalmol 49:895

Miyata K, Nagamoto T, Maruoka S, Tanabe T, Nakahara M, Amano S (2002) Efficacy and safety of the soft-shell technique in cases with a hard lens nucleus. J Cataract Refract Surg 28:1546–1550

Myers WG, Shugar JK (2009) Optimizing the intracameral dilation regimen for cataract surgery: prospective randomized comparison of 2 solutions. J Cataract Refract Surg 35:273–276

Nuyts RMMA, Edelhauser HF, Holley GP (1995) Intraocular irrigating solutions: a comparison of Hartmann's lactated Ringer's solution, BSS and BSS Plus. Graefes Arch Clin Exp Ophthalmol 233:655–661

Ozturk R, Osher R (2006) Capsular staining: recent developments. Curr Opin Ophthalmol 17:42–44

Phillipall B, Crandall AS, Mamalis N, Olson RJ (1997) Intraoperative miotics and posterior capsular opacification following phacoemulsification with intraocular lens insertion. Ophthalmic Surg Lasers 28:911–914

Powe NR, Schein OD, Gieser SC et al (1994) Synthesis of the literature on visual acuity and complications following cataract extraction with intraocular lens implantation. Cataract Patient Outcome Research Team. Arch Ophthalmol 112:239–252

Puckett TR, Peele KA, Howard RS et al (1995) Intraocular irrigating solutions: a randomized clinical trial of balanced salt solution plus and dextrose bicarbonate lactated ringer's solution. Ophthalmology 102:291–296

Ravalico G, Botteri E, Baccara F (2003) Long-term endothelial changes after implantation of anterior chamber intraocular lenses in cataract surgery. J Cataract Refract Surg 29:1918–1923

Rebolleda G, Munoz-Negrete FJ, Gutierrez-Ortiz C (2001) Topical plus intracameral lidocaine versus retrobulbar anesthesia in phacotrabeculectomy: prospective randomized study. J Cataract Refract Surg 27:1214–1220

Rhee SW, Youn DH, Lee J, Choi O, Jung HR (1987) The effects of Bendaline on cataracts. Korean J Ophthalmol 1:31–37

Schutz JS, Mavrakanas NA (2010) What degree of anaesthesia is necessary for intraocular surgery? It depends on whether surgery is "open" or "closed". Br J Ophthalmol 94(10):1400–1413

Sekundo W, Dick HB, Schmidt JC (2004) Lidocaine-assisted xylocaine jelly anesthesia versus one quadrant sub-Tenon infiltration for self-sealing sclero-corneal incision routine phacoemulsification. Eur J Ophthalmol 14:111–116

Shaw AD, Ang GS, Eke T (2007) Phacoemulsification complication rates. Ophthalmology 114:2101–2102

Shen H, Gou S, Shen J, Zhu Y, Zhang Y, Chen X (2010) Synthesis and biological evaluations of novel bendazac lysine analogues as potent anticataract agents. Bioorg Med Chem Lett 20:2115–2118

Storr-Paulsen A, Norregaard JC, Farik G, Tarnhoj J (2007) The influence of viscoelastic substances on the corneal endothelial cell population during cataract surgery: a prospective study of cohesive and dispersive viscoelastics. Acta Ophthalmol Scand 85:183–187

Swamy MS, Abraham EC (1989) Inhibition of lens crystallin glycation and higher molecular weight aggregate formation by aspirin in vitro and in in vivo. Invest Opththalmol Vis Sci 30:1120–1126

Testa M, Iuliano G, Marino E, Buongiovanni C, Forgione A, Paolercio F, Russo G, Trapanese A, Morton P (1987) Higher efficacy of flunoxaprofen over bendazac and other nonsteroidal anti-inflammatory drugs in the treatment of cataracts. Curr Ther Res 42(1):182–189

Timucin OB, Karadag MF, Aslanci ME, Baykara M (2016) Methylene blue-related corneal edema and iris discoloration. Arq Bras Oftalmol 79(2):121–122

Umapathy A, Donaldson P, Lim J (2013) Antioxidant delivery pathways in the anterior eye. Biomed Res Int 2013:1–10

Van Heyningen R, Harding JJ (1986) Do aspirin-like analgesics protect against cataract? A case–control study. Lancet 1:1111–1113

Varma SD, Hegde KR, Kovtun S (2010) Inhibition of selenite-induced cataract by caffeine. Acta Ophthalmol 88:e245–e249

Vasavada V, Dixit NV, Raj SM et al (2009) Comparison between Ringer's lactate and balanced salt solution on postoperative outcomes after phacoemulsification: a randomized clinical trial. Indian J Ophthalmol 57:191–195

Vazirani J, Basu S (2013) Role of topical, subconjunctival, intracameral, and irrigative antibiotics in cataract surgery. Curr Opin Ophthalmol 24(1):60–65

Waddell KM, Reeves BC, Johnson GI (2004) A comparison of anterior and posterior chamber lenses after cataract extraction in rural Africa: a within patient randomized trial. Br J Ophthalmol 88:734–739

Wagle AA, Wagle AM, Bacsal K et al (2007) Practice preferences of ophthalmic anaesthesia for cataract surgery in Singapore. Singapore Med J 48:287–290

Wegener A, Laser-Junga H (2009) Photography of the anterior eye segment according to Scheimpflug's principle: options and limitations – a review. Clin Experiment Ophthalmol 37:144–154

Williams DL, Muunday P (2006) The effect of a topical antioxidant formulation including N-acetylcarnosine on canine cataract: a preliminary study. Vet Ophthalmol 9:311–316

World Health Organization (2013) Universal eye health: a global action plan 2014-2019. http://www.who.int/blindness/AP2014_19_English.pdf

Zhao L, Chen XJ, Zhu J et al (2015) Lanosterol reverses protein aggregation in cataracts. Nature 523:607–611

Glaucoma-Intraocular Pressure Reduction

Alex S. Huang, Lilit Minasyan, and Robert N. Weinreb

Contents

Abstract

Medical treatment is a mainstay for the management of glaucoma (Realini 2011; Marquis and Whitson 2005; Hoyng and van Beek 2000). Intraocular pressure (IOP) lowering has been long recognized as and still represents the primary and most widely employed treatment to prevent glaucomatous vision loss (Musch et al. 2011; Leske et al. 2003; The Advanced Glaucoma Intervention Study (AGIS) 2000). Soon after the recognition that "tension" or IOP was related to glaucoma, pharmacological agents were introduced in the mid-1800s, first with

A.S. Huang • L. Minasyan
Doheny Eye Centers, Doheny and Stein Eye Institutes, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA

R.N. Weinreb (✉)
Hamilton Glaucoma Center, Shiley Eye Institute, UCSD, La Jolla, CA, USA
e-mail: Rweinreb@ucsd.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_24

the calabar bean (Realini 2011; Proudfoot 2006). Since then, an explosion of pharmacological agents targeting numerous intracellular and molecular signaling pathways has resulted in a plethora of drugs to lower IOP and treat glaucoma. Aqueous humor dynamics provides the basis for understanding each of these medical therapies.

Keywords
Aqueous humor outflow • Aqueous humor production • Glaucoma • Intraocular pressure (ICP) • Pharmacology

1 Introduction

Medical treatment is a mainstay for the management of glaucoma (Realini 2011; Marquis and Whitson 2005; Hoyng and van Beek 2000). Intraocular pressure (IOP) lowering has been long recognized as and still represents the primary and most widely employed treatment to prevent glaucomatous vision loss (Musch et al. 2011; Leske et al. 2003; The Advanced Glaucoma Intervention Study (AGIS) 2000). Soon after the recognition that "tension" or IOP was related to glaucoma, pharmacological agents were introduced in the mid-1800s, first with the calabar bean (Realini 2011; Proudfoot 2006). Since then, an explosion of pharmacological agents targeting numerous intracellular and molecular signaling pathways has resulted in a plethora of drugs to lower IOP and treat glaucoma. Aqueous humor dynamics provides the basis for understanding each of these medical therapies.

A common starting point for understanding IOP-related pharmacology is the Goldman equation or one of its many variations. Simply stated, IOP is predicted as $\mathrm{IOP} = F(R) + \mathrm{EVP}$ (Brubaker 2004). Each variable of the Goldman equation has a structural counterpart (Fig. 1). In this way, physiology is related to anatomy. F relates to aqueous humor production occurring in the ciliary processes. From here, aqueous humor flows into the posterior chamber and then between the lens and iris into the anterior chamber (AC). From the AC two outflow routes exist. Aqueous humor that exits through the traditional outflow pathway flows through the trabecular meshwork (TM) of the angle draining into Schlemm's canal (SC). Aqueous humor then flows through collector channels into an intrascleral venous plexus before passing into aqueous and episcleral veins returning the fluid to the systemic venous circulation (Johnson 2006; Ashton 1951). As per the Goldman equation, for the traditional outflow pathway, R predominately relates to TM resistance although post-trabecular contributions do exist (Johnson 2006). EVP quantifies the far distal back pressure in the episcleral veins that can reflect retrograde to the eye also to increase IOP (Sit et al. 2011). To account for the uveoscleral (second) outflow pathway, the Goldman equation is expanded to $\mathrm{IOP} = [F(\mathrm{in}) - F(u)] \times R + \mathrm{EVP}$ (Larsson). This modified equation best models uveoscleral outflow $[F(u)]$ as bulk fluid outflow from the eye that mostly behaves in a pressure-independent fashion (hence its inclusion in the F as opposed to R variable).

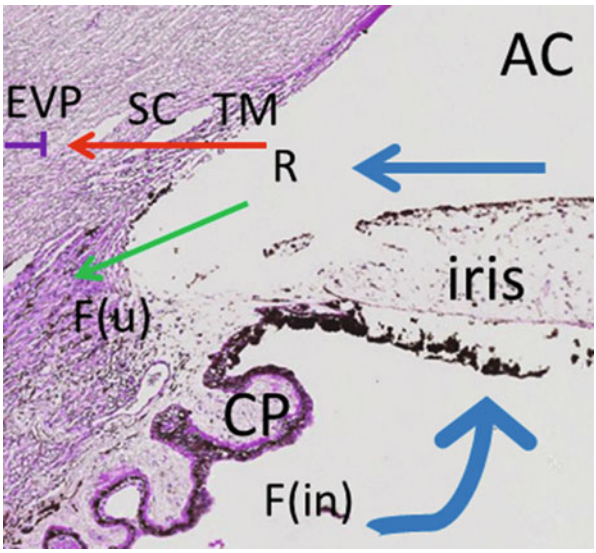

Fig. 1 Aqueous humor outflow. IOP = [*F*(in) − *F*(*u*)] × *R* + EVP. Each variable in the Goldman equation has an anatomical counterpart. Aqueous humor production [*F*(in)] starts at the ciliary processes (CP), and aqueous humor travels (*blue arrows*) into the anterior chamber (AC). Two outflow pathways exist with the conventional pathway (*red arrow*; *R*) leading to episcleral veins (EVP; *purple line*). The uveoscleral pathway (*green arrow*) and bulk outflow is best modeled as a part of *F* [*F*(u)]. *IOP* intraocular pressure, *TM* trabecular meshwork, *SC* Schlemm's canal

Table 1 summarizes glaucoma pharmacology agents highlighting prominent benefits and disadvantages of each class. Details for each of the drug classes follow in the chapter.

2 Aqueous Production Suppressant Agents

2.1 Beta-Blockers

2.1.1 History

Discovered by Phillips et al. in 1967, propanolol was the first beta-blocker to show that intraocular pressure was reduced in glaucoma patients with systemic administration of the drug for systemic hypertension (Phillips et al. 1967) Within 1 year, there were topical formulations of propranolol available that reduced IOP. The drug was not successful as a topical agent, however, because of its local corneal anesthetic (membrane-stabilizing) properties as well as an adverse effect on tear production. Other beta-blockers were studied subsequently that had additional limitations, such as profound dry eye syndrome, subconjunctival fibrosis, and tachyphylaxis. Ultimately, timolol, a beta-blocker with no membrane-stabilizing activity, was introduced to the market in 1976. It was approved for general use by

Table 1 Summary of intraocular pressure lowering agents

	Advantages	Disadvantages
Aqueous suppressants		
Beta-blockers		
Timolol	Substantial literature; comes in combo format	Side effects (asthma, bradycardia, etc.)
Betaxolol	Better for patients with asthma/ cardiac problems	Lessened IOP reduction
Carteolol	Improved lipids	Similar to timolol
Carbonic anhydrase inhibitors		
Acetazolamide	Substantial literature	Side effects (renal stress, blood dyscrasia, ocular surface burning, etc.)
Methazolamide	Less renal stress	Lessened IOP response compared to acetazolamide
Dorzolamide	Substantial literature; comes in combo format	Not as strong as oral CAI with lessened but persistent side effects
Brinzolamide	Better pH than dorzolamide	Similar to dorzolamide
Alpha-adrenergic agonists		
Epinephrine	Few advantages	Side effects (adrenochrome, arrhythmias, pupillary dilation etc.)
Dipiverfin	Better corneal penetration than epinephrine	Side effects (similar to epinephrine)
Clonidine	Fewer side effects than epi/dipiverfin	Systemic hypotension
Apraclonidine	Fewer side effects than clonidine	Tachyphylaxis and conjunctivitis
Brimonidine	Higher alpha-2 affinity than apraclonidine; comes in combo format	Same tachyphylaxis and conjunctivitis
Outflow agents		
Conventional outflow		
Parasympathomimetics	Substantial literature on conventional outflow drugs	Side effects are multiple; decreases uveoscleral outflow
ROCK inhibitors	Investigational	Hyperemia, investigational
Uveoscleral outflow		
Prostaglandins	Large IOP Reduction	Side effects (lash growth, iris pigmentation, hyperemia, uveitis, etc.)
	Lesser side effects	
	Convenient dosing	

the FDA in 1978 and has had a prominent role in the treatment of glaucoma ever since.

2.1.2 Mechanism

Mechanistically, beta-blockers reduce aqueous humor production. Epithelial cells of the ciliary body express both beta1 and beta2 adrenergic receptors (beta2 receptors comprise 75–90%). Catecholamine stimulation of beta adrenergic receptors leads to the activation of cAMP, a second messenger involved in the activation of protein kinase A, which results in the production and secretion of aqueous humor in an energy-dependent manner. Beta-blockers are synthetic organic molecules that inhibit this binding and thus the action of endogenous catecholamines (Grieshaber and Flammer 2010).

2.1.3 Drug Effects and Formulation

Timolol is a nonselective beta-blocker and lowers IOP by 20–35% on average (Zimmerman and Kaufman 1977; Wilson et al. 1979). The effect happens within 1 h of instillation and can be present for up to 4 weeks after discontinuation. It is available in 0.25% and 0.5% concentrations, used once to twice daily. A formulation with a gel vehicle is approved for once-daily application. As a result of its binding to iris melanin, and losing some of its efficacy, patients with a dark iris may need a higher concentration (Katz and Berger 1979; Salminen et al. 1985).

In a study investigating both the diurnal and nocturnal efficacy of timolol, Liu and colleagues measured IOP in patients treated either with timolol or latanoprost over 24-hour period and showed that during nocturnal periods timolol had no impact on IOP reduction from baseline while latanoprost significantly lowered baseline IOP (Liu et al. 2004; Liu et al. 2009). Therefore, when used once daily, morning administration of timolol is preferred in order to minimize ocular vascular hypotension during the overnight IOP rise.

Extended use of timolol and all beta-blockers may have reduced effectiveness secondary to tachyphylaxis from the constant exposure to a drug agonist (long-term drift). Similarly, receptor saturation and drug-induced up-regulation of beta receptors may also occur within a few weeks with loss of effectiveness (short-term escape). As such, it has been reported that after 2 years, about 50% of patients treated initially with timolol monotherapy require different or additional medication, or temporarily discontinuing timolol (“drug holiday”) for effective IOP control (Kobelt et al. 1998).

Betaxolol is a beta1-selective adrenoreceptor antagonist that also lowers IOP by inhibiting aqueous humor production. Betaxolol is available as a 0.25% suspension and 0.5% solution that are each administered twice daily. Since the ciliary body contains less beta1 than beta2 adrenoreceptors, it is thought that betaxolol may lower IOP by its weaker beta2 blocking properties explaining its smaller IOP reduction (18–26%) (Stewart et al. 1986; Radius 1983). As betaxalol has less beta2 blockage than a nonselective beta-blocker, and beta2 blockade can lead to bronchoconstriction, it may be useful for IOP reduction in patients with pulmonary disorders. Similarly, but somewhat as a surprise, betaxolol can also be considered in

patients with cardiac disorders. Despite the expectation that beta1 selective blockade would influence both cardiac ionotropy and chronotropy, post-marketing surveillance with betaxolol has suggested fewer cardiac events. Hypotheses for this include increased plasma protein binding to reduce free drugs levels in the blood.

Carteolol is a nonselective B-adrenergic antagonist with intrinsic sympathomimetic activity (ISA), meaning that there is also weak partial agonist activity. Its efficacy is comparable to timolol with average IOP reduction of 20–32% (Scoville et al. 1988; Stewart et al. 1991). It is available in 1% and 2% solutions and is applied twice daily. Due to its ISA, carteolol produces an early transient beta-adrenoreceptor agonist response, which was hoped to be protective against some of the systemic adverse effects seen with other beta-blockers such as decreased heart rate and exercise induced dyspnea. While this has not proven to be the case (Schnarr 1988; Flury et al. 1986), carteolol has been shown to result in better serum lipid profiles compared to timolol (Freedman et al. 1993). In a comparative study involving topical carteolol, there was an associated 3.3% decrease in plasma HDL and a 4% increase in total cholesterol/HDL-C ratio, while timolol produced changes of 8% and 10%, respectively (Shaw and Weinreb 1991).

Other nonselective beta blocking agents are levobunolol and metipranolol. They both lower IOP by inhibiting aqueous humor production. Levobunolol is available in 0.25% and 0.5% solutions and is typically administered twice daily. Metipranolol is available in 0.1%, 0.3%, and 0.6% solutions and is also administered twice daily. Levobunolol and metipranolol IOP reduction is similar to timolol (Geyer et al. 1988; The Levobunolol Study Group 1985; Krieglstein et al. 1987; Schmitz-Valckenberg et al. 1984; Bleckmann et al. 1983). Metipranolol has also been associated with granulomatous anterior uveitis (Akingbehin and Villada 1991).

Apart from topical delivery, glaucoma patients are often elderly and may have co-existing systemic comorbidities requiring systemic beta-blockers. A prospective multicenter randomized and double-masked 12-month clinical trial demonstrated that patients with concomitant systemic beta-blocker therapy combined with topical timolol had 19% IOP reduction from baseline at peak, as opposed to greater (~25%) IOP reduction in patients not on systemic beta-blockers (Schuman 2000). Also, the patients treated with concurrent topical and systemic beta-blockers experienced greater reduction in heart rate compared to the patients receiving only local therapy. Therefore, simultaneous use of systemic beta-blocker therapy may reduce the efficacy of topical beta-blockers and compromise safety as well.

2.1.4 Adverse Effects

In general, topical nonselective β-adrenoceptors are effective and well tolerated by most patients. Reported local adverse effects include conjunctival hyperemia, burning, stinging, superficial punctuate keratitis, and worsening dry eye symptoms secondary to reduction of tear flow (McMahon et al. 1979; Van Buskirk 1980; Coakes et al. 1981).

Contrary to their favorable ocular side effect profile, they may induce severe systemic side effects by blocking beta1 and beta2 adrenoreceptors in the heart and lungs. The most important possible side effects for nonselective agents are:

- Bradycardia, congestive heart failure, syncope, atrioventricular block (McMahon et al. 1979; Van Buskirk 1980; Fraunfelder 1980; Nelson et al. 1986)
- Bronchoconstriction, which can be life threatening in patients with asthma or COPD (McMahon et al. 1979; Van Buskirk 1980; Fraunfelder 1980; Nelson et al. 1986; Diggory et al. 1995; Sadiq et al. 1998)
- Masking of hypoglycemia in diabetics (Velde and Kaiser 1983)
- Modification of lipid metabolism with increase in serum triglycerides and decreased in serum HDL (Freedman et al. 1993).
- Reduction in exercise endurance, possibly secondary to reduced cardiac contractility
- Central nervous system effects such as anxiety, depression, sexual impotence, fatigue, disorientation, and confusion (McMahon et al. 1979; Van Buskirk 1980; Fraunfelder 1980)

The use of nasolacrimal occlusion or eyelid closure decreases systemic uptake via the nasopharyngeal epithelium and decreases the plasma levels by up to 70%; it also increases intraocular uptake, thereby minimizing the unwanted systemic side effects (Zimmerman et al. 1992).

2.2 Carbonic Anhydrase Inhibitors (CAI)

2.2.1 History

Oral acetazolamide has been in clinical use as an ocular hypotensive agent since 1954 following Bernard Becker's observation of its potent IOP-lowering effect. Unfortunately, extraocular actions resulted in a number of unpleasant and unsafe side effects often resulting in discontinuation of therapy (Pfeiffer 1997). For the next 30–40 years there was an unsuccessful search for topical CAIs that would effectively lower IOP without the systemic side effects. In retrospect, the requirement of about 90–100% enzyme activity inhibition in the ciliary epithelium was a difficult hurdle. Dorzolamide was the first topical CAI that was introduced to market in 1995 after FDA approval. It was both water and lipid soluble with good corneal penetrance and was highly effective at inhibiting carbonic anhydrase. Plus, its systemic side effect profile was much more favorable than that of systemically dosed CAIs. Several years after dorzolamide was released, another topical CAI, brinzolamide, received FDA approval (Realini 2011).

2.2.2 Mechanism

Mechanistically, CAIs decrease aqueous humor production (Sugrue 2000). Carbonic anhydrase itself generates $Na+$ and $HCO3-$ ions, allowing water to enter the ciliary epithelial cells, to facilitate aqueous humor production. CAIs are small

molecules that inhibit aqueous humor formation by direct inhibition of carbonic anhydrase isoenzyme II in the ciliary epithelium and perhaps to a lesser extent, by production of generalized acidosis with systemic administration. There are different isoenzymes also present in corneal endothelium, iris, retinal pigment epithelium, red blood cells, brain, and kidney.

2.2.3 Drug Effects and Formulations

Systemic CAIs can be given orally, intramuscularly, or intravenously. Systemically, acetazolamide and methazolamide are typically used with the former being more common. Acetazolamide is often used in acute settings such as acute angle closure-glaucoma, in short-term use in patients on maximum medical management as a temporizing measure before surgery, or temporally post-laser or incisional surgery. Oral CAIs begin to act within 1 h of administration with maximum effect at 2–4 h. Sustained-release acetazolamide can reach its maximum effect within 3–6 h of administration. The onset of action for intravenous acetazolamide is within 2 min, with peak effect at 15 min. The maximum effect on IOP is reached with acetazolamide 250 mg four times daily or 500 mg sustained-release capsules twice daily. The IOP reduction is about 20–30% but can be dependent on the initial IOP.

Methazolamide has a longer half-life, is less bound to serum protein, and undergoes first pass hepatic metabolism, reducing the overall risk of adverse events compared to acetazolamide. To the contrary, acetazolamide is not metabolized and is excreted in urine. The IOP reducing effect of methazolamide is somewhat smaller than what is seen with acetazolamide (Dahlen et al. 1978). The recommended dosage for methazolamide is 25, 50, or 100 mg 3 times daily. Therefore, methazolamide and acetazolamide may be preferred in those with renal and liver disease, respectively.

Topical CAIs currently marketed are dorzolamide 2%, used 3 times daily and brinzolamide 1%, used 3 times daily. Dorzolamide has a relatively more acidic pH of 5.5 as opposed to brinzolamide (pH 7.4). In a 1-year multicenter study, dorzolamide as monotherapy lowered IOP by 23%. This was similar to betaxolol but slightly lower than the reduction of IOP achieved by timolol (26%) (Strahlman et al. 1995). In one study, there was no statistically significant difference between dorzolamide 2% used 3 times daily versus 2 times daily (Lippa et al. 1992).

Dorzolamide has been shown to be less effective at lowering IOP than oral acetazolamide in a comparative study with timolol as a first line drug (Maus et al. 1997). It is additive in hypotensive efficacy to timolol even though they both lower IOP by inhibiting aqueous humor production (Boyle et al. 1998). Comparisons between 2% dorzolamide and 0.2% brimonidine as a monotherapy showed similar efficacy at both peak and trough (Stewart et al. 2000; Whitson et al. 2004).

The IOP-lowering effect of brinzolamide is similar to that of dorzolamide. In one meta-analysis on the efficacy of several ocular hypotensive agents, brinzolamide reduced IOP by 17% from baseline at both peak and trough while dorzolamide reduced IOP by 22% at peak (van der Valk et al. 2005). In patients already receiving latanoprost monotherapy, adding brinzolamide to the treatment had an

IOP-lowering efficacy during the nocturnal period as opposed to timolol, whereas both timolol and brinzolamide significantly reduced IOP during daytime periods (Liu et al. 2009). For patients on an adequate oral CAI dose, there does not appear to be an advantage to also using topical CAI.

2.2.4 Adverse Effects

Adverse effects of systemic CAI therapy are usually dose-related. Well-known adverse effects are paresthesias of the hands and feet, nausea, vomiting, diarrhea, unpleasant taste in mouth, fatigue, and weight loss, as well as depression (Pfeiffer 1997). There is an increased risk of the formation of calcium oxalate and calcium phosphate renal stones with acetazolamide as opposed to methazolamide because of its renal clearance. CAIs are chemically derived from sulfa drugs and this may cause allergic reactions and cross reactivity to similar sulfa drugs. Aplastic anemia, thrombocytopenia, and agranulocytosis can also occur, although very rare, and routine complete blood counts are not recommended (Fraunfelder et al. 1985). Rarely, Steven–Johnson syndrome and exfoliative dermatitis may occur during treatment with oral acetazolamide and methazolamide. Systemic acidosis, hypokalemia, and hyponatremia can result from inhibition of carbonic anhydrase in the kidney, and concurrent use of other drugs that causes potassium loss such as thiazides should be avoided with serum potassium monitoring in patients with chronic use.

Topical CAIs are devoid of most of the systemic adverse effects seen with oral CAIs. The local adverse effects of dorzolamide are stinging, burning, itching, and tearing. Ocular surface irritation with brinzolamide is less than that with dorzolamide presumably secondary to its more physiologic pH of 7.4. Stinging and burning was noted in 16.4% of patients on dorzolamide 3 times daily versus 3% of patients on brinzolamide three times daily (Silver 1998). Eyes with compromised endothelial function may be at risk for corneal decompensation with topical CAI use (Konowal et al. 1999). Blurred vision, fatigue, and bitter taste have also been reported. This is probably due to drug enrichment in tears draining into the oropharynx and inhibition of carbonic anhydrase present in the saliva and taste buds (Supuran and Scozzafava 2000). Also, blurring is more common with brinzolamide as opposed to dorzolamide as it is a suspension (Carta et al. 2012).

2.3 Adrenergic Agonists

2.3.1 History

Epinephrine was the first adrenergic agonist used in the treatment of glaucoma in the form of subconjunctival injections in the 1920s after Frenchman Jean Darier found its IOP reducing effect while studying adrenal extracts. However, it was not widely used as its IOP lowering was variable and there were adverse side effects such as cardiac arrhythmias, pupillary dilation, and eye lid retraction from alpha1 adrenergic receptor activation. Dipiverfin then took advantage of corneal esterases converting dipiverfin to epinephrine. With ~17 times better ocular penetration, a

lower concentration (0.1%) of dipiverfin was needed compared with epinephrine (1–2%) (Mandell et al. 1978). Although there were fewer side effects, many of them remained, so that the search of new systemic or topical formulations continued.

The first alpha2 preferential adrenergic agonist was clonidine. Clonidine demonstrated not only IOP-lowering effect but also caused systemic hypotension (CNS-mediated adrenergic effect) secondary to its lipophilicity and penetration of the blood–brain barrier. As such, while available as a topical agent in Europe, clonidine has never been approved in the USA.

2.3.2 Mechanism

Mechanism of action of this class is broad. Primarily, most alpha-adrenergic agonists reduce IOP by acutely decreasing aqueous humor production (measured by fluorophotometry) ~20% with contralateral effects of ~12%. After cessation of the drug and resolution of dampened aqueous humor production, continued IOP reduction can be seen and is attributed to enhanced (~5×) uveoscleral outflow through elevation of prostaglandin levels. Influences on uveoscleral outflow of some of these agents place them in a unique class of beneficially decreasing aqueous production while increasing outflow. Moreover, potential direct and IOP-independent neuroprotective roles have been proposed and discussed later in this chapter.

2.3.3 Drug Effects and Formulation

In the 1980s, a second-generation alpha2 adrenergic agonist, apraclonidine, was introduced. Its IOP-lowering effect was discovered by chance when it was being tested as an agent for controlling hemorrhage after Nd:YAG laser iridotomies (Realini 2011). It was found to have no effect on controlling bleeding but had significant effect on reducing post-laser IOP spikes. Compared to clonidine, apraclonidine hydrochloride (para-aminoclonidine) is more selective for alpha2 receptors and is more hydrophilic because of the addition of an amide group. This limits its transport through the blood brain barrier and risk of systemic hypotension. However, because of tachyphylaxis and a high rate of conjunctivitis (Araujo et al. 1995), today apraclonidine (0.5–1%) is now mostly reserved for controlling short-term IOP spikes following surgery or laser procedures.

Brimonidine (0.2%, approved for 3 times daily use in the USA) is a 3rd generation alpha2 adrenoreceptor agonist and was introduced in 1996. It has significantly higher alpha2 adrenergic receptor agonist affinity (23- to 32-fold compared to apraclonidine) with little effect on systemic blood pressure, making it the preferred chronic alpha2 adrenergic agonist for glaucoma treatment today (Burke and Schwartz 1996). Brimonidine's peak IOP reduction is approximately 20–30% (2 h postdose) (Walters 1996; Schuman 1996; Schuman et al. 1997; Derick et al. 1997). At peak, brimonidine 0.2% used twice daily is comparable to timolol 0.5% twice daily and superior to betaxolol 0.25% used twice daily. However, at trough (12 h postdose) it is significantly less effective than timolol (only 14–15% IOP reduction), but is comparable to betaxolol (Schuman 1996; Schuman et al. 1997; Serle 1996). A large meta-analysis comparing latanoprost 0.005%

and brimonidine 0.2% found that latanoprost is more effective at IOP reduction in subjects with normal tension glaucoma, ocular hypertension, and POAG for up to 1 year after initiation of therapy (Fung et al. 2007). Also, brimonidine has significant effect on IOP reduction during diurnal time points but has no nocturnal IOP-lowering effect (Liu et al. 2010).

2.3.4 Adverse Effects

Long-term administration of modern alpha agonists has been mostly limited by its allergic and ocular surface reactions. Blepharitis, blepharoconjunctivitis, or conjunctivitis has been observed in about 9% of patients, conjunctival follicles in 7.8%, mild hyperemia in 26.3%, staining of the cornea in 8%, blurred vision, and foreign body sensation in 17% which can occur either occasionally or chronically although many of these effects may take years to manifest (Schuman 1996; Schuman et al. 1997). To this point, brimonidine 0.15% and 0.1% has been reformulated in purite as an alternative preservative in an attempt to lower ocular surface allergies. The theoretical advantage of purite is that, compared to benzalkonium chloride (BAK), in the presence of light, oxygen, and water, purite dissociates into sodium and chloride ions with a neutral pH. While similar in IOP-lowering efficacy, the purite-containing preparations have been shown to have fewer ocular surface side effects (Cantor et al. 2009).

It must be noted that the use of brimonidine is contraindicated in infants and young children, particularly those less than 6 years of age or weighing less than 20 kg because of serious life threatening side effects such as seizures, bradycardia, hypotension, hypotonia, apnea, dyspnea, hypoventilation, cyanosis, and lethargy (Zimmerman 1997).

3 Traditional Aqueous Humor Outflow Agents

Traditional aqueous humor outflow focused on the TM, SC, and their relationship. Seminal work by Morton Grant not only showed that the TM was the primary resistor to aqueous humor outflow in the eye, but also that this resistance was elevated in the glaucomatous eye (Grant 1963). The primary means to modify this traditional pathway outflow resistance has been through parasympathomimetic agents now followed by rho-kinase inhibitors that are under study.

The ciliary muscle has three portions (longitudinal, circular, and radial) with muscular contraction under parasympathomimetic control (Tamm et al. 1992). The longitudinal muscle inserts into the scleral spur that is familiar to the clinical ophthalmologist under gonioscopic view as a white band under the TM band and directly above the ciliary body band (Fig. 2). As such, with contraction of the longitudinal muscle, the scleral spur is pulled down and away from the TM leading to a greater opening and decreased outflow resistance (Hartridge 1925).

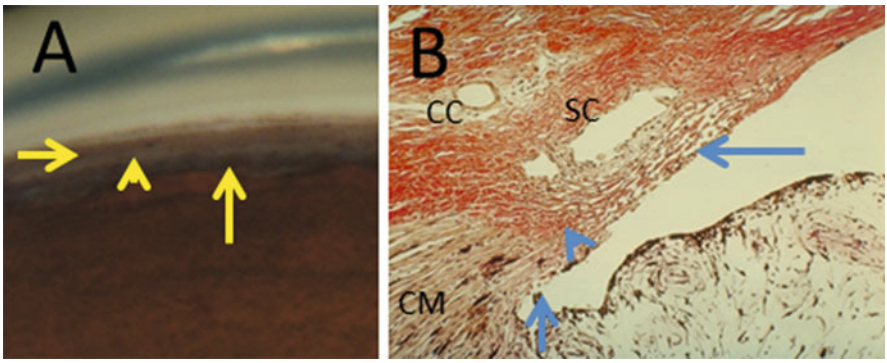

Fig. 2 Conventional aqueous humor outflow. (**a**) Gonioscopic view of the angle. Note how the trabecular meshwork (TM; *horizontal arrow*) sits above the scleral spur (*arrowhead*) which then rests above the ciliary body band (*vertical arrow*). (**b**) Likewise in histological section (picrosirius red stain for collagen), the TM (*horizontal arrow*) lies above the scleral spur (*arrowhead*) that lies above the ciliary body band (*vertical arrow*) (courtesy of AFIP through Dr. Narsing Rao). Therefore, in both images one can imagine how contraction of the ciliary muscle leads to downward deflection of the scleral spur and stretching open the TM. *SC* Schlemm's canal, *CC* collector channel, *CM* ciliary muscle

3.1 Parasympathomimetics

3.1.1 History

The Nigerian calabar bean was introduced in the 1800s by Sir Thomas Fraser and Argyll Robertson as the first IOP-lowering medicine (Realini 2011; Proudfoot 2006). Unbeknownst to users, the calabar bean eventually became a source for extracting physostigmine.

3.1.2 Mechanism

Today, parasympathomimetic agents fall into two general classes, direct and indirect. Direct agents such as pilocarpine act by direct stimulation of muscarinic receptors on ocular muscles to cause contraction. Indirect agents, like physostigmine, instead inhibit acetylcholinesterase to elevate endogenous acetylcholine levels at the motor endplate to cause the same effects. With either direct or indirect activation, the result is miosis with the pupillary sphincter muscle, accommodation with the circular component of the *ciliary* muscle, and decreased TM outflow resistance by contracting the *longitudinal* ciliary muscle in the eye.

3.1.3 Drug Effects and Formulations

Pilocarpine is mostly commonly prescribed and found 0.5–8%, typically used QID, and available as a 4% gel for nighttime use. IOP reduction is modest at 20–30% (Drance and Nash 1971). Contradictory effects on uveoscleral outflow are described (see uveoscleral outflow section). Phospholine iodide is a short-acting indirect agent that is not always generally available. Carbachol uniquely works by

both stimulating muscarinic receptors and inhibiting acetylcholinesterase (O'brien and Swan 1941).

3.1.4 Adverse Effects

While effective in lowering IOP, parasympathomimetics have several important adverse effects. Parasympathomimetic activation induces miosis that can affect patient vision, ciliary muscle contraction that causes myopic shifts (Poinoosawmy et al. 1976), brow aches, and risk of retinal detachment (Pape and Forbes 1978) – particularly in individuals with myopia. Additionally, systemic effects relating to manipulating the autonomic nervous system exist such as bradycardia, diarrhea, and bronchospasm (O'brien and Swan 1941). Specific to the eye, a cataractogenic effect and uveitic inflammation have been seen (Thoft 1968). Importantly, one must be careful with indirect parasympathomimetic agents as prolonged respiratory suppression can be seen in cases of concurrent use of succinylcholine for short-term paralysis prior to endotracheal intubation (Ellis and Esterdahl 1967). Succinylcholine works as a nicotinic acetylcholine receptor agonist to create a persistent and paralyzing depolarization of the muscle endplate. In the presence of an acetylcholinesterase inhibitor to further elevate endogenous acetylcholine, the paralyzing depolarization of succinylcholine is reversed more slowly and with greater difficulty.

3.2 Rho-Kinase Inhibitors

3.2.1 History

Implicated in IOP regulation in the early 2000s (Honjo et al. 2001), Rho-kinase (ROCK) proteins are serine/threonine kinases that are regulated by Rho family proteins. Rho proteins are a group of small phosho-guanosine binding proteins whose activity are controlled by the identity of the bound phospho-nucleotide (GTP = active; GDP = inactive) (Wang and Chang 2014). Downstream from ROCK, many kinase substrates exist. Phosphorylation of LIM kinase stabilizes actin filaments through cofilin, and phosphorylation of myosin light chain kinase causes contraction of actin fibers (Wang and Chang 2014). Together, in the eye, these effects are proposed to stabilize and contract actin in TM cells to therefore increase outflow resistance.

3.2.2 Mechanism

As such, ROCK *inhibitors* are hypothesized to alter actin homeostasis and cell morphology to improve aqueous humor outflow (Wang and Chang 2014). In experimental models, ROCK inhibitor Y-27632 led to 80% increased outflow through the TM with decreased myosin light chain phosphorylation and increased extracellular spaces near the juxtacanalicular tissue (Rao et al. 2001). With topical application of Y-27632 or alternative analogues in rabbit eyes, a measured reduction in outflow facility was found with approximately 30–46% reduction in IOP (Honjo et al. 2001).

3.2.3 Drug Effects and Formulations

Clinically in humans, multiple agents are in investigational trials (Wang and Chang 2014) with doses that range from 0.003% to 0.1% either with BID or daily dosing. However, recent press releases by Aerie Pharmaceuticals reported phase 3 results (ROCKET 1) demonstrating IOP lowering but failure to meets the primary endpoint of non-inferiority compared to beta-blockers.

3.2.4 Adverse Effects

In investigational studies, the most commonly seen adverse effect was conjunctival hyperemia. This was not unexpected given that the ROCK protein is known to cause vasoconstriction through cytoskeletal effects (Loirand et al. 2006). Subconjunctival hemorrhages have been noted.

3.3 Uveoscleral Outflow Agents

3.3.1 History

When Anders Bill introduced radio-labeled (I^{125}) albumin into the eyes of macaque monkeys, he could only recover approximately half of the radioactivity from the scleral surface or in the periphery (Bill 1965) which represented the ultimate extension of the traditional outflow pathway. This suggested a second outflow pathway with the missing fraction of radioactivity found in the uveal tract now known to be the uveoscleral outflow pathway.

The discovery of the uveoscleral outflow pathway was facilitated by a series of serendipitous observations. The original uveoscleral outflow experiments were conducted on the eyes of young monkeys. First, typically with radioactivity-based methods, significant species-specific differences in uveoscleral outflow have been found with monkeys showing greatest uveoscleral outflow as a percentage of total outflow (50%) (Bill 1971), with dogs and humans in the middle (~15%) (Bill and Phillips 1971), and animals such as cats and rabbits (~3%) (Bill 1989; Bill 1966) at the rear. Simultaneously, using a series of indirect methods to calculate uveoscleral outflow from measurable components of the Goldman equation, age was also found to be an important factor with younger humans and monkeys showing up to 40% increased uveoscleral outflow (Bill 1965; Bill and Phillips 1971; Toris et al. 1999). Therefore, the initial choice of young monkeys by Anders Bill facilitated the discovery of the uveoscleral outflow pathway.

With the stratification of greater or lessen uveoscleral outflow across a continuum of species and ages, natural conditions were created (young vs old; or monkey vs rabbit) that could allow for studying the anatomical correlates that influenced uveoscleral outflow. The uveoscleral outflow pathway itself represents aqueous humor bulk flow entering the ciliary muscle leading to the supraciliary space into the choroid and suprachoroidal clefts subsequently leaving the eye via the perivascular spaces of the emissarial scleral channels or directly through the collagen bundles of the sclera itself under the conjunctiva (Fig. 3). Comparing monkey eyes to rabbit eyes, the monkey ciliary muscle demonstrated intermuscular

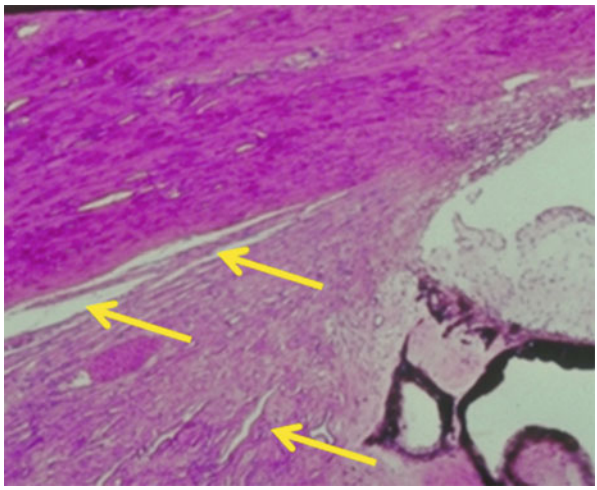

Fig. 3 Uveoscleral outflow pathway. (**a**) The young human ciliary body demonstrates clefts (*yellow arrows*) important for uveoscleral outflow. Courtesy of AFIP from Dr. Narsing Rao

spaces absent in the rabbit ciliary muscle which were instead filled with hyaluronan (Lutjen-Drecoll et al. 1990). In parallel to this, young human and monkey ciliary muscles showed increased intermuscular spaces that were then absent and filled with extracellular matrix material in older eyes (Tamm et al. 1992; Lutjen-Drecoll et al. 1988). As such, species and age data suggested that the large intermuscular spaces in the ciliary muscle represented the starting point of uveoscleral outflow for aqueous humor from the anterior chamber and may be the rate-limiting step. Additionally, in glaucoma dogs (Barrie et al. 1985) and ocular hypertensive humans, ciliary muscle sheaths and tendons were thickened with deposition of plaque-like presentations leading to muscle fiber fusion suggesting a potential pathological role in the ciliary muscle and uveoscleral outflow in disease as well (Lutjen-Drecoll et al. 1986).

3.3.2 Mechanism

Focused on the ciliary muscle, the pharmacology of the uveoscleral outflow pathway can be divided into two themes of (a) muscular contraction and (b) extracellular matrix (ECM) regulation. The ciliary muscle is normally under parasympathetic control with pilocarpine (a parasympathomimetic agonist) and atropine (a parasympathomimetic antagonist), respectively, contracting and relaxing the various components of the ciliary muscle (longitudinal, circular, and radial). The relationship between the parasympathomimetic pathway and the different components of the ciliary muscle therefore creates a unique paradox respective to *total* aqueous humor outflow. As described above, parasympathomimetic activation (pilocarpine) contracts the *longitudinal* (Hartridge 1925) ciliary muscle of the traditional outflow pathway leading to opening of the TM, decreased outflow resistance (Bartels and Neufeld 1980), and measured decreased IOP. In contrast, parasympathomimetic activation also contracts the *circular* ciliary muscle, but in

this case effectively abolishes (Barany and Rohen 1965) the intermuscular spaces important for uveoscleral outflow which can theoretically increase IOP. Not surprisingly, Anders Bills observed decades ago that parasympathomimetic activation blocked uveoscleral outflow in a small number of humans (Bill and Phillips 1971). In eyes scheduled to be removed for choroidal melanomas, uveoscleral outflow was measured with a radioactive method under no treatment, pilocarpine, or atropine. Uveoscleral outflow was noted to be 4–14% of total outflow in untreated patients, 0–3% in pilocarpine treated patients, and 4–27% in atropine treated patients (Bill and Phillips 1971). Nevertheless, considering the parasympathomimetic pathway alone, the effect of pilocarpine on the TM must be more pronounced than that on the uveoscleral pathway alone because there is an overall lowering of intraocular pressure when pilocarpine solely is topically applied to the eye (Davanger 1964). This balance becomes even more interesting when combining parasympathomimetic manipulation with prostaglandin-mediated ECM and uveoscleral outflow alterations discussed below.

Prostaglandins and ECM modification have been the primary tool to modify uveoscleral outflow and today is first line treatment for glaucoma. All of the critical components of the prostaglandin pathway are present in the ciliary body. First, in situ hybridization, immunohistochemistry, or binding studies using a labeled parent molecule of PGF2α have shown both prostaglandin EP and FP receptors in the ciliary muscle (Ocklind et al. 1996; Csukas et al. 1993). This is important as receptor subtypes influence prostaglandin binding and possibly ocular hypotensive responses to various agents (see below).

The ECM and related regulatory proteins are found in the ciliary muscle. The ECM of the ciliary muscle is composed of collagen (subtypes I, III, IV, and VI), fibronectin, elastin, and laminins (Weinreb et al. 1994). Matrix metalloproteinases (MMPs) are also found in the uveoscleral outflow pathway. MMPs are a family of neutral proteases that degrade various extracellular matrix proteins in a peptide sequence-specific manner (Murphy and Docherty 1992). Importantly, many MMPs such as MMP 1, 2, 3, and 9 are known to be controlled through an AP-1 transcriptional regulatory element in their promoters (Lindsey and Weinreb 1998). Using ciliary muscle cultures, prostaglandins can stimulate C-Fos, a DNA-binding protein that activates AP-1 containing genes, to activate MMP expression (Lindsey et al. 1994). Simultaneously, elevated MMP protein levels and enzyme activity have been shown by immunohistochemistry and zymographic studies, respectively, in ciliary muscle in response to prostaglandins (Weinreb et al. 1997; Gaton et al. 2001). Then with PFG2α, topical administration has been shown to widen intermuscular spaces in the ciliary muscle (Stjernschantz et al. 1998) that while latanoprost did not replicate, latanoprost topical administration did diminish ECM protein levels in the ciliary muscle (Ocklind 1998). Therefore, mechanistically, prostaglandins are proposed to activate C-Fos through prostaglandin receptors in the uveoscleral outflow pathway to increase expression of MMPs to decrease ciliary muscle ECM and/or create widened spaces important for uveoscleral outflow.

Studied in humans, prostaglandin analogues have been shown to increase uveoscleral outflow. Derived from PGF2α, with initial studies based on analogue

PhXA34, initial reports demonstrated a 100% increase in uveoscleral outflow in 22 normal and ocular hypertensive patients using an indirect method with fluorometrically derived outflow facility (based on changing aqueous flow parameter before and after aqueous suppressants), an estimated episcleral venous pressure, and applanated IOP (Toris et al. 1993). Importantly, the once-daily dosing, slower onset of actions, and delayed intraocular pressure recovery after cessation agree with a mechanism of action that requires time for changes to gene expression and extracellular matrix remodeling.

3.3.3 Drug Effects and Formulations

Currently available prostaglandin formulations include latanoprost (0.005%), bimatoprost (0.01 or 0.03%), travoprost (0.004%), and tafluprost (0.0015%). Latanoprost was first introduced in the USA in 1996 and soon became the most widely prescribed drug for glaucoma. Not only was latanoprost found to reduce IOP better than timolol (Camras 1996), but latanoprost was found to be equally effective at reducing IOP at night and day (Orzalesi et al. 2000). These agents mostly lowered IOP approximately 30% with greater efficacy seen with once-daily nighttime dosing as opposed to twice-daily dosing. One large multi-centered trial (Parrish et al. 2003) showed equal efficacy among latanoprost, bimatoprost, and travoprost as single agents. Most studies show superiority of these prostaglandin analogues over aqueous suppressants. The various formulations vary by receptor specificity (travoprost FP$>>$EP specific, latanoprost FP$>$EP, bimatoprost EP$=$FP) (Stjernschantz et al. 1998). This may explain subtle differences in the drugs, and why patients who have become tolerated to one prostaglandin analogue (latanoprost) can still demonstrate IOP lowering in response to another (bimatoprost) (De Moraes et al. 2015). As such, clinically with a potent IOP-lowering effect in a friendly once-daily dosing regimen, prostaglandin analogues serve as a first-line medical therapeutic for IOP lowering in glaucoma.

The future of uveoscleral pharmacology touches upon drug delivery ideas such as drug impregnated punctal plugs, intracameral delivery, or new formulations. Latanoprostene-bunod chemically combines a prostaglandin analogue with a nitric oxide (NO) donor which can in parallel improve traditional outflow (Weinreb et al. 2014). A phase 2b trial has suggested greater IOP lowering with latanoprostene-bunod than with latanoprost alone (Weinreb et al. 2014).

3.3.4 Adverse Effects

Adverse effects with prostaglandin analogues are rare with mild conjunctival hyperemia (3–15%) being the most common. Other adverse effects include ocular surface discomfort (burning/stinging/tearing) (Camras 1996; Watson and Stjernschantz 1996), rare ocular inflammation (Camras et al. 1996) with possibly cystoid macular edema (mostly in cases associated with complicated lens extraction or previous maculopathy) (Hoyng et al. 1997), and iris pigmentation changes that are really most significant in cases of unilateral use in people with light irises (Camras et al. 1996). Increase in eye lash length with chronic use represents an interesting adverse event that has become an additional novel application for these

drugs. More recently, observations have been made regarding orbital and globe positional changes (prostaglandin periorbitopathy) with long-term prostaglandin analogue use (Custer and Kent 2015).

3.4 Distal Outflow

Traditionally, anterior segment glaucoma pathology has been ascribed to the traditional outflow pathway with a heavy emphasis on TM disease. However, multiple lines of evidence now point to the distal portions of the traditional outflow pathway as being more than just passive conduits for fluid flow (Hariri et al. 2014), possibly contributing to the disease process in glaucoma, and potentially having a role in glaucoma therapeutics.

Both historical and modern evidence point toward potential roles for the distal outflow pathway in glaucoma disease and management. While Morton Grant's original work highlighted the role of the TM in normal and diseased eyes, careful examination of his data and subsequent work showed that while the TM is the primary resistor of the eye, approximately 30% of outflow resistance resides past the TM (Grant 1963; Van Buskirk 1977). Plus, in parallel to elevation of TM resistance in glaucoma (~3-fold), post-TM contributions to resistance also elevated (~2.5 fold) to nearly the same extent as that of the diseased TM itself suggesting that the resistance problem in glaucoma was a whole eye instead of a sole TM problem.

These historical notes agree with modern day evidence. Most anterior segment molecular theories for elevated IOP involve some pathologic agent (TGF-beta, CTGF, etc.) in the aqueous humor (Braunger et al. 2015). Since elevation of outflow resistance in glaucoma is not absolute (meaning zero outflow), some flow still continues past the TM in the diseased state into the distal outflow pathway potentially negatively impacting these regions as well. Therefore, a framework for disease beyond the TM exists. Furthermore, the explosion of trabecular-targeted (ablation or bypass) Minimally Invasive Glaucoma Surgeries also suggests that glaucoma is more involved than just the TM. Real but variable success of TM bypass/ablation (Minckler et al. 2008; Craven et al. 2012) points toward still undetermined complexities in aqueous humor outflow beyond the TM in the distal outflow pathway.

While no known pharmacological agent has been proven to directly affect the distal outflow pathway, anecdotal reports exist. Various reports of increased steroid-response from post-operative steroids after trabecular-targeted MIGS suggest continued points of regulation past the TM (Harvey and Khaimi 2011). Not surprisingly, nitrates can affect the episcleral veins which then could theoretically influence IOP.

Ultimately, to fully understand the role of and serve as a platform to develop agents to target the distal outflow pathway, one must be able to visualize the full outflow pathway. Today, aqueous angiography represents the only real-time, comprehensive, and physiologic outflow pathway imaging technique (Saraswathy et al. 2016). Comprehensive means that information of outflow is obtained

360 degrees around the limbus simultaneously and incorporates contributions from the anterior chamber all the way through the episcleral vein. Aqueous angiography in enucleated animal and human eyes demonstrated segmental outflow that both may influence glaucoma surgical success (through placement) and provide a method to screen for pharmacological agents that effect the traditional outflow pathway (both proximal and distal) in the future.

4 Combination Drugs

Medications that are combined in a single bottle offer an additional benefit of improved compliance and convenience (Sverrisson et al. 1999), improved efficacy, reduced cost, less exposure to preservatives such as BAK (Hughes et al. 2005), and elimination for the potential of "wash-out effects" (Boyle et al. 1998). While first line therapy typically consists of monotherapy, for patients requiring more than one topical IOP-lowering agent for adequate control, it is very common to use combined medications.

The first combination drop available in the USA was, a fixed combination of timolol maleate 0.5% and dorzolamide 2% used twice daily which demonstrated similar efficacy compared to two agents given separately (ranging from 27 to 33%) (Boyle et al. 1998). Another combination treatment for glaucoma initially approved in Canada and Europe, and then later approved in the USA was a twice-daily combination of timolol maleate 0.5% and brimonidine 0.2% which has been demonstrated to be superior to monotherapy using either agent alone in reducing IOP (Craven et al. 2005; Larsson 2001). Interestingly, there have been no additive effects with regard to adverse events. To the contrary, timolol/brimonidine combination has shown even lower incidence of ocular allergies compared to brimonidine monotherapy alone (Motolko 2008).

The above-mentioned available fixed combinations contain timolol, which can limit their use in patients with comorbidities that contraindicate beta blockade. Also, by creating fixed combination containing beta-blockers, the nighttime component of BID dosing may be potentially problematic given beta-blocker lack of IOP reduction efficacy at night (see beta-blockers, above). Therefore, a fixed combination of brinzolamide 1% and brimonidine 0.2% was recently approved for three times daily use in the USA and twice daily in Europe. Unsurprisingly, twice-daily brinzolamide/brimonidine was more effective than both brinzolamide and brimonidine alone and non-inferior to that of concomitant therapy with brinzolamide plus brimonidine (Aung et al. 2014; Gandolfi et al. 2014). Tolerability and adverse event profile was similar to that of its individual components (Gandolfi et al. 2014).

Fixed combination products have been developed for each of the PGAs presently in clinical use (latanoprost, bimatoprost, and travoprost) and timolol 0.5% but are not currently approved in the USA.

5 Neuroprotection

As the pathologic hallmark of glaucomatous damage is the loss of retinal ganglion cells, all pharmacologic treatments ultimately relate to neuroprotection of these cells. While not traditionally considered neuroprotective, IOP reduction is in it of itself neuroprotection since glaucoma progression is mitigated. Nevertheless, the search for a primary agent to directly influence the health and survivability of ganglion cells in the retina via direct posterior-segment targeted drug action apart from IOP has been ongoing (Vasudevan et al. 2011; Chang and Goldberg 2012).

Despite the fact that betaxolol's is less effective in lowering IOP than a nonselective beta-blocker, a few reports have suggested better visual field preservation with long-term betaxolol therapy compared to timolol implying possible neuroprotection (Collignon-Brach 1992; Messmer et al. 1991; Kaiser et al. 1992). One hypothesis for this maybe calcium channel antagonistic effects resulting in enhanced retinal and optic nerve head blood flow (Hoste and Sys 1994; Hoste 1998). Alternatively, betaxolol may block excessive Ca2+ and Na+ ions influx into neurons in response to different injuries (Osborn et al. 2005). Better designed clinical trials will be necessary to prove a betaxolol neuroprotective effect. Additionally, one can speculate that timolol treatment was instead detrimental. In fact, the Low-pressure Glaucoma Treatment Study (LOGTS) study showed that timolol compared to brimonidine treated patients were more likely to have HVF progression (Krupin et al. 2011).

Brimonidine has been demonstrated in numerous experimental studies to be neuroprotective (WoldeMussie et al. 2001). First, brimonidine clearly enters the eye, and alpha-adrenergic receptors reside in the retina (Matsuo and Cynader 1992) so that key components for theoretical brimonidine-mediated neuroprotection are present. Interestingly, as mentioned above, the LOGTS showed that low-pressure glaucoma patients treated with brimonidine (who did not develop ocular allergy since study had over 40% drop-out rate secondary to ocular allergy/adverse events) were less likely to have visual field progression than patients treated with timolol (Krupin et al. 2011). Otherwise strong supporting clinical evidence has been sparse.

Targeting retinal glutamate excitotoxicity represents a pure posterior-segment neuroprotective concept. Unfortunately, while experimental models using *N*-methyl-D-aspartate (NMDA) receptor antagonism with MK-801 conferred neuroprotection (Hare et al. 2001), unpublished clinical trials with Memantine (a NMDA receptor antagonist) were not successful per report in meeting primary endpoints or in showing real difference from placebo for glaucoma treatment.

6 Summary

The unifying concept for glaucoma pharmacology revolves around the Goldman equation which outlines the anatomical contributors to aqueous humor outflow and inflow. However, as with the case of any initial mathematical modeling of physiology, the Goldman equation is also due for an update given the explosion of

knowledge regarding the full outflow pathways. In the future, an expanded Goldman equation incorporating distal outflow and better modeling of uveoscleral outflow (Sit 2015) will provide a more comprehensive paradigm for organizing pharmacological concepts.

Moving forward, ophthalmology already has a significant number of aqueous suppressants and uveoscleral outflow agents. After the introduction of parasympathomimetic agents, there has been a dearth of primary conventional outflow drugs despite a large emphasis on TM research over the decades. Conventional outflow may be a greater target in the future. The potential for posterior-segment targeted neuroprotective agents is also promising but early. Thus future and best practice methods for treating glaucoma and mitigating vision loss will eventually likely be comprised by a combination of both anterior IOP-related and posterior-targeted approaches.

References

Akingbehin T, Villada J (1991) Metipranolol associated granulomatous anterior uveitis. Br J Ophthalmol 75:519–523

Araujo SV, Bond JB, Wilson RP et al (1995) Long term effect of apraclonidine. Br J Ophthalmol 79:1098–1101

Ashton N (1951) Anatomical study of Schlemm's canal and aqueous veins by means of neoprene casts. Part I. Aqueous veins. Br J Ophthalmol 35:291–303

Aung T, Laganovska G, Hernandez Paredes TJ et al (2014) Twice-daily brinzolamide/brimonidine fixed combination versus brinzolamide or brimonidine in open-angle glaucoma or ocular hypertension. Ophthalmology 121:2348–2355

Barany E, Rohen J (1965) Localized contraction and relaxation within the ciliary muscle of the vervet monkey (Cercopithecus ethiops). In: Rohen J (ed) The structure of the eye, second symposium. FK Schattauer Verlag, Stuttgart, pp 287–311

Barrie KP, Gum GG, Samuelson DA, Gelatt KN (1985) Quantitation of uveoscleral outflow in normotensive and glaucomatous Beagles by 3H-labeled dextran. Am J Vet Res 46:84–88

Bartels SP, Neufeld AH (1980) Mechanisms of topical drugs used in the control of open angle glaucoma. Int Ophthalmol Clin 20:105–116

Bill A (1965) The aqueous humor drainage mechanism in the cynomolgus monkey (Macaca irus) with evidence for unconventional routes. Invest Ophthalmol 4:911–919

Bill A (1966) The routes for bulk drainage of aqueous humour in rabbits with and without cyclodialysis. Doc Ophthalmol 20:157–169

Bill A (1971) Aqueous humor dynamics in monkeys (Macaca irus and Cercopithecus ethiops). Exp Eye Res 11:195–206

Bill A (1989) Uveoscleral drainage of aqueous humor: physiology and pharmacology. Prog Clin Biol Res 312:417–427

Bill A, Phillips CI (1971) Uveoscleral drainage of aqueous humour in human eyes. Exp Eye Res 12:275–281

Bleckmann H, Pham Duy T, Grajewski O (1983) Therapeutic efficacy of metipranolol eye drops 0.3% versus timolol eye drops 0.25%: a double blind cross over study. In: Merte HJ (ed) Metipranolol. Springer, Wien

Boyle JE, Ghosh K, Gieser D et al (1998) A randomized trial comparing the dorzolamide–timolol combination given twice daily to monotherapy with timolol and dorzolamide. Ophthalmology 105:1945–1951

Braunger BM, Fuchshofer R, Tamm ER (2015) The aqueous humor outflow pathways in glaucoma: a unifying concept of disease mechanisms and causative treatment. Eur J Pharm Biopharm 95:173–181

Brubaker RF (2004) Goldmann's equation and clinical measures of aqueous dynamics. Exp Eye Res 78:633–637

Burke J, Schwartz M (1996) Preclinical evaluation of brimonidine. Surv Ophthalmol 41:S9–S18

Camras CB (1996) Comparison of latanoprost and timolol in patients with ocular hypertension and glaucoma: a six-month masked, multicenter trial in the United States. The United States Latanoprost Study Group. Ophthalmology 103:138–147

Camras CB, Alm A, Watson P, Stjernschantz J (1996) Latanoprost, a prostaglandin analog, for glaucoma therapy. Efficacy and safety after 1 year of treatment in 198 patients. Latanoprost Study Groups. Ophthalmology 103:1916–1924

Cantor L, Liu C, Batoosing A, Hollander D (2009) Safety and tolerability of brimonidine purite 0.1% and brimonidine 0.15%: a meta-analysis of two phase 3 studies. Curr Med Res Opin 25:1615–1620

Carta F, Supuran CT, Scozzafava A (2012) Novel therapies for glaucoma: a patent review 2007–2011. Expert Opin Ther Pat 22:79–88

Chang EE, Goldberg JL (2012) Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement. Ophthalmology 119:979–986

Coakes RL, Mackie IA, Seal DV (1981) Effects of long-term treatment with timolol on lacrimal gland function. Br J Ophthalmol 65:603–605

Collignon-Brach J (1992) Long-term effect of ophthalmic betaadrenoceptor antagonists on intraocular pressure and retinal sensitivity in primary open-angle glaucoma. Curr Eye Res 11:1–3

Craven ER, Walters TR, Williams R, Chou C, Cheetham JK, Schiffman R (2005) Combigan Study Group. Brimonidine and timolol fixed-combination therapy versus monotherapy: a 3-months randomised trial in patients with glaucoma or ocular hypertension. J Ocul Pharm Ther 21:337–348

Craven ER, Katz LJ, Wells JM, Giamporcaro JE (2012) Cataract surgery with trabecular microbypass stent implantation in patients with mild-to-moderate open-angle glaucoma and cataract: two-year follow-up. J Cataract Refract Surg 38:1339–1345

Csukas S, Bhattacherjee P, Rhodes L, Paterson CA (1993) Prostaglandin E2 and F2 alpha binding sites in the bovine iris ciliary body. Invest Ophthalmol Vis Sci 34:2237–2245

Custer PL, Kent TL (2015) Observations on prostaglandin orbitopathy. Ophthal Plast Reconstr Surg 32:102–105

Dahlen K, Epstein DL, Grant WM et al (1978) A repeated dose-response study of methazolamide in glaucoma. Arch Ophthalmol 96:2214–2218

Davanger M (1964) The pressure-reducing effect of miotics in glaucoma simplex and in normal eyes, elucidated by hydrodynamic calculations based on Poiseulle's law. Acta Ophthalmol (Copenh) 42:773–781

De Moraes G, Susanna R, Germano R, Susanna C, Milena C (2015) Effect of switching from latanoprost to bimatoprost in primary open-angle glaucoma patients who experienced a hypotensive reduction during treatment. American Glaucoma Society, San Diego

Derick RJ, Robin AL, Walters TR et al (1997) Brimonidine tartrate: a one-month dose response study. Ophthalmology 104:131–136

Diggory P, Cassels-Brown A, Vail A et al (1995) Avoiding unsuspected respiratory side-effects of topical timolol with cardioselective or sympathomimetic agents. Lancet 345:1604–1606

Drance SM, Nash PA (1971) The dose response of human intraocular pressure to pilocarpine. Can J Ophthalmol 6:9–13

Ellis PP, Esterdahl M (1967) Echothiophate iodide therapy in children. Effect upon blood cholinesterase levels. Arch Ophthalmol 77:598–601

Flury H, TournouxT A, Martenet AC (1986) Tolerance and pharmacologic effectiveness of antiglaucoma eyedrops. Klin Monatsbl Augenheilkd 188:573–575

Fraunfelder FT (1980) Interim report: national registry of possible drug-induced ocular side effects. Ophthalmology 87:87–90
Fraunfelder FT, Meyer SM, Bagby GC Jr et al (1985) Hematologic reactions to carbonic anhydrase inhibitors. Am J Ophthalmol 15:79–81
Freedman SF, Freedman NJ, Shields MB et al (1993) Effects of ocular carteolol and timolol on plasma high-density lipoprotein cholesterol level. Am J Ophthalmol 116:600–611
Fung AT, Reid SE, Jones MP et al (2007) Meta-analysis of randomised controlled trials comparing latanoprost with brimonidine in the treatment of open-angle glaucoma, ocular hypertension or normal-tension glaucoma. Br J Ophthalmol 91:62–68
Gandolfi SA, Lim J, Sanseau AC et al (2014) Randomized trial of brinzolamide/brimonidine versus brinzolamide plus brimonidine for open-angle glaucoma or ocular hypertension. Adv Ther 31:1213–1227
Gaton DD, Sagara T, Lindsey JD, Gabelt BT, Kaufman PL, Weinreb RN (2001) Increased matrix metalloproteinases 1, 2, and 3 in the monkey uveoscleral outflow pathway after topical prostaglandin F(2 alpha)-isopropyl ester treatment. Arch Ophthalmol 119:1165–1170
Geyer O, Lazar M, Novack GD et al (1988) Levobunolol compared with timolol: a four-year study. Br J Ophthalmol 72:892–896
Grant WM (1963) Experimental aqueous perfusion in enucleated human eyes. Arch Ophthalmol 69:783–801
Grieshaber MC, Flammer J (2010) Is the medication used to achieve the target intraocular pressure in glaucoma therapy of relevance? An exemplary analysis on the basis of two beta-blockers. Prog Retin Eye Res 29:79–93
Hare W, WoldeMussie E, Lai R, Ton H, Ruiz G et al (2001) Efficacy and safety of memantine, an NMDA-type open-channel blocker, for reduction of retinal injury associated with experimental glaucoma in rat and monkey. Surv Ophthalmol 45 (Suppl 3): S284–S289; discussion S95–6
Hariri S, Johnstone M, Jiang Y, Padilla S, Zhou Z et al (2014) Platform to investigate aqueous outflow system structure and pressure-dependent motion using high-resolution spectral domain optical coherence tomography. J Biomed Opt 19:106013
Hartridge H (1925) Helmholtz's theory of accommodation. Br J Ophthalmol 9:521–523
Harvey BJ, Khaimi MA (2011) A review of canaloplasty. Saudi J Ophthalmol 25:329–336
Honjo M, Tanihara H, Inatani M, Kido N, Sawamura T et al (2001) Effects of rho-associated protein kinase inhibitor Y-27632 on intraocular pressure and outflow facility. Invest Ophthalmol Vis Sci 42:137–144
Hoste AM (1998) Ca2+ channel blocking activity of propranolol and betaxolol in isolated bovine retinal microartery. J Cardiovasc Pharmacol 32:390–396
Hoste AM, Sys SU (1994) The relaxant action of betaxolol on isolated bovine retinal microarteries. Curr Eye Res 13:483–487
Hoyng PF, van Beek LM (2000) Pharmacological therapy for glaucoma: a review. Drugs 59:411–434
Hoyng PF, Rulo AH, Greve EL, Astin M, Gjötterberg M (1997) Fluorescein angiographic evaluation of the effect of latanoprost treatment on blood-retinal barrier integrity: a review of studies conducted on pseudophakic glaucoma patients and on phakic and aphakic monkeys. Surv Ophthalmol 41(Suppl 2):S83–S88
Hughes BA, Bacharach J, Carven ER (2005) A three-month, multicenter, double-masked study of the safety and efficacy of travoprost 0.004%/timolol 0.5% ophthalmic solution compared to travoprost 0.004% ophthalmic solution and timolol 0.5% dosed concomitantly in subjects with open angle glaucoma or ocular hypertension. J Glaucoma 14:392–399
Johnson M (2006) What controls aqueous humour outflow resistance? Exp Eye Res 82:545–557
Kaiser HJ, Flammer J, Messmer C et al (1992) Thirty month visual field follow up of glaucoma patients treated with beta blockers. J Glaucoma 1:153
Katz IM, Berger ET (1979) Effects of iris pigmentation on response of ocular pressure to timolol. Surv Ophthalmol 23:395–398

Kobelt G, Jönsson L, Gerdtham U, Krieglstein GK (1998) Direct costs of glaucoma management following initiation of medical therapy: a simulation model based on an observational study of glaucoma treatment in Germany. Graefes Arch Clin Exp Ophthalmol 236:811–821

Konowal A, Morrison JC, Brown SV et al (1999) Irreversible corneal decompensation in patients treated with topical dorzolamide. Am J Ophthalmol 127:403–406

Krieglstein GK, Novack GD, Voepel E et al (1987) Levobunolol and metipranolol: comparative ocular hypotensive efficacy, safety, and comfort. Br J Ophthalmol 71:250–253

Krupin T, Liebmann JM, Greenfield DS, Ritch R, Gardiner S, Low-Pressure Glaucoma Treatment Study Group (2011) A randomized trial of brimonidine versus timolol in preserving visual function: results from the Low-Pressure Glaucoma Treatment Study. Am J Ophthalmol 151:671–681

Larsson LI (2001) Aqueous humor flow in normal human eyes and treated with brimonidine and timolol alone and in combination. Arch Ophthalmol 119:492–495

Larsson L-I, Alm A (1998) Clinical aspects of uveoscleral outflow. In: Alm A, Kaufman PL, Kitazawa Y, Lutjen-Drecoll E, Stjernschantz J, Weinreb RN (eds) Uveoscleral outflow: biology and clinical aspects. Mosby-Wolfe, London, pp 73–86

Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L et al (2003) Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. Arch Ophthalmol 121:48–56

Lindsey JD, Weinreb RN (1998) Effects of prostaglandins on uveoscleral outflow. In: Alm A, Kaufman PL, Kitazawa Y, Lutjen-Drecoll E, Stjernschantz J, Weinreb RN (eds) Uveoscleral outflow: biology and clinical aspects. Mosby-Wolfe, London, pp 41–56

Lindsey JD, To HD, Weinreb RN (1994) Induction of c-fos by prostaglandin F2 alpha in human ciliary smooth muscle cells. Invest Ophthalmol Vis Sci 35:242–250

Lippa EA, Carlson L-I, Ehinger B, Eriksson L-O, FinnstroÈ MK et al (1992) Dose response and duration of action of dorzolamide, a topical carbonic anhydrase inhibitor. Arch Ophthalmol 110:495–499

Liu J, Kirpke DF, Weinreb R (2004) Comparison of the nocturnal effects of once-daily timolol and latanoprost on intraocular pressure. Am J Ophthalmol 138:389–395

Liu JH, Medeiros FA, Slight JR, Weinreb RN (2009) Comparing diurnal and nocturnal effects of brinzolamide and timolol on intraocular pressure in patients receiving latanoprost monotherapy. Ophthalmology 116:449–454

Liu L, Medeiros F, Slight J, Weinreb R (2010) Diurnal and nocturnal effects of brimonidine monotherapy on intraocular pressure. Ophthalmology 117:2075–2079

Loirand G, Guérin P, Pacaud P (2006) Rho kinases in cardiovascular physiology and pathophysiology. Circ Res 98:322–334

Lutjen-Drecoll E, Shimizu T, Rohrbach M, Rohen JW (1986) Quantitative analysis of 'plaque material' between ciliary muscle tips in normal- and glaucomatous eyes. Exp Eye Res 42:457–465

Lutjen-Drecoll E, Tamm E, Kaufman PL (1988) Age changes in rhesus monkey ciliary muscle: light and electron microscopy. Exp Eye Res 47:885–899

Lutjen-Drecoll E, Schenholm M, Tamm E, Tengblad A (1990) Visualization of hyaluronic acid in the anterior segment of rabbit and monkey eyes. Exp Eye Res 51:55–63

Mandell AI, Stentz F, Kitabchi AE (1978) Dipivalyl epinephrine: a new pro-drug in the treatment of glaucoma. Ophthalmology 85:268–275

Marquis RE, Whitson JT (2005) Management of glaucoma: focus on pharmacological therapy. Drugs Aging 22:1–21

Matsuo T, Cynader MS (1992) Localization of alpha-2 adrenergic receptors in the human eye. Ophthalmic Res 24:213–219

Maus TL, Larsson LI, McLaren JW et al (1997) Comparison of dorzolamide and acetazolamide as suppressors of aqueous humor flow in humans. Arch Ophthalmol 115:45–49

McMahon CD, Shaffer RN, Hoskins HD Jr et al (1979) Adverse effects experienced by patients taking timolol. Am J Ophthalmol 88:736–738

Messmer C, Flammer J, Stumpfig D (1991) Influence of betaxolol and timolol on the visual fields of patients with glaucoma. Am J Ophthalmol 112:678–681
Minckler D, Mosaed S, Dustin L, Ms BF (2008) Trabectome (trabeculectomy-internal approach): additional experience and extended follow-up. Trans Am Ophthalmol Soc 106:149–159, discussion 59–60
Motolko M (2008) Comparison of allergy rates in glaucoma patients receiving brimonidine 0.2% monotherapy versus fixed-combination brimonidine 0.2% –timolol 0.5% therapy. Curr Med Res Opin 24:2663–2667
Murphy G, Docherty AJ (1992) The matrix metalloproteinases and their inhibitors. Am J Respir Cell Mol Biol 7:120–125
Musch DC, Gillespie BW, Niziol LM, Lichter PR, Varma R; CIGTS Study Group (2011) Intraocular pressure control and long-term visual field loss in the Collaborative Initial Glaucoma Treatment Study. Ophthalmology 118:1766–1773
Nelson WL, Fraunfelder FT, Sills JM et al (1986) Adverse respiratory and cardiovascular events attributed to timolol ophthalmic solution, 1978-1985. Am J Ophthalmol 102:606–611
O'brien CS, Swan KC (1941) Doryl in the treatment of glaucoma simplex. Trans Am Ophthalmol Soc 39:175–193
Ocklind A (1998) Effect of latanoprost on the extracellular matrix of the ciliary muscle. A study on cultured cells and tissue sections. Exp Eye Res 67:179–191
Ocklind A, Lake S, Wentzel P, Nister M, Stjernschantz J (1996) Localization of the prostaglandin F2 alpha receptor messenger RNA and protein in the cynomolgus monkey eye. Invest Ophthalmol Vis Sci 37:716–726
Orzalesi N, Rossetti L, Invernizzi T, Bottoli A, Autelitano A (2000) Effect of timolol, latanoprost, and dorzolamide on circadian IOP in glaucoma or ocular hypertension. Invest Ophthalmol Vis Sci 41:2566–2573
Osborn NN, Wood JP, Chidlow G (2005) Invited review: neuroprotective properties of certain beta-adrenoreceptor antagonists used for the treatment of glaucoma. J Ocul Pharmacol 21:175–181
Pape LG, Forbes M (1978) Retinal detachment and miotic therapy. Am J Ophthalmol 85:558–566
Parrish RK, Palmberg P, Sheu WP; XLT Study Group (2003) A comparison of latanoprost, bimatoprost, and travoprost in patients with elevated intraocular pressure: a 12-week, randomized, masked-evaluator multicenter study. Am J Ophthalmol 135:688–703
Pfeiffer N (1997) Dorzolamide: development and clinical application of a topical carbonic anhydrase inhibitor. Surv Ophthalmol 42:137–151
Phillips CI, Howitt G, Rowlands DJ (1967) Propanolol as ocular hypotensive agent. Br J Ophthalmol 51:222–226
Poinoosawmy D, Nagasubramanian S, Brown NA (1976) Effect of pilocarpine on visual acuity and on the dimensions of the cornea and anterior chamber. Br J Ophthalmol 60:676–679
Proudfoot A (2006) The early toxicology of physostigmine: a tale of beans, great men and egos. Toxicol Rev 25:99–138
Radius RL (1983) Use of betaxolol in the reduction of elevated intraocular pressure. Arch Ophthalmol 101:898–900
Rao PV, Deng PF, Kumar J, Epstein DL (2001) Modulation of aqueous humor outflow facility by the Rho kinase-specific inhibitor Y-27632. Invest Ophthalmol Vis Sci 42:1029–1037
Realini T (2011) A history of glaucoma pharmacology. Optom Vis Sci 88:36–38
Sadiq SA, Fielding K, Vernon SA (1998) The effect of timolol drops on respiratory function. Eye 12:386–389
Salminen L, Imre G, Huupponen R (1985) The effect of ocular pigmentation on intraocular pressure response to timolol. Acta Ophthalmol 173:15–18
Saraswathy S, Tan JCH, Francis BA, Hinton DR, Weinreb RN, Huang AS (2016) Aqueous angiography: a real-time, physiologic, and comprehensive aqueous humor outflow imaging technique. PLoS One 25, e0147176

Schmitz-Valckenberg P, Jonas J, Bambring DF (1984) Reductions in pressure with metipranolol 0.1%. Z Prakt Augenhkd 5:171–175
Schnarr KD (1988) Comparative multicenter study of carteolol eyedrops with other beta blockers in 768 patients under normal conditions. Klin Monatsbl Augenheilkd 192:167–172
Schuman JS (1996) Clinical experience with brimonidine 0.2% and timolol 0.5% in glaucoma and ocular hypertension. Surv Ophthalmol 41(Suppl 1):S27–S37
Schuman J (2000) Effects of systemic beta-blocker therapy on the efficacy and safety of topical brimonidine and timolol. Brimonidine Study Groups 1 and 2. Ophthalmology 107:1171–1177
Schuman JS, Horwitz B, Choplin NT et al (1997) A 1-year study of brimonidine twice daily in glaucoma and ocular hypertension: a controlled, randomized, multicenter clinical trial (Chronic Brimonidine Study Group). Arch Ophthalmol 115:847–852
Scoville B, Mueller B, White BG, Krieglstein GK (1988) A double-masked comparison of carteolol and timolol in ocular hypertension. Am J Ophthalmol 105:150–154
Serle JB (1996) A comparison of the safety and efficacy of twice daily brimonidine 0.2% versus betaxolol 0.25% in subjects with elevated intraocular pressure: the Brimonidine Study Group III. Surv Ophthalmol 41:S39–S47
Shaw B, Weinreb R (1991) Topical timolol decreases plasma high-density lipoprotein cholesterol level (Letter). Arch Ophthalmol 109:1341–1342
Silver LH (1998) Clinical efficacy and safety of brinzolamide (Azopt), a new topical carbonic anhydrase inhibitor for primary open-angle glaucoma and ocular hypertension. Am J Ophthalmol 126:400–408
Sit A (2015) Do we need a new Goldman equation. American Glaucoma Society, San Diego
Sit AJ, Ekdawi NS, Malihi M, McLaren JW (2011) A novel method for computerized measurement of episcleral venous pressure in humans. Exp Eye Res 92:537–544
Stewart RH, Kimbrough RL, Ward RL (1986) Betaxolol vs timolol: a six-month double-blind comparison. Arch Ophthalmol 104:46–48
Stewart WC, Shields MB, Allen RC et al (1991) A 3-month comparison of 1% and 2% carteolol and 0.5% timolol in open-angle glaucoma. Graefes Arch Clin Exp Ophthalmol 229:258–261
Stewart WC, Sharpe ED, Harbin TS et al (2000) Brimonidine 0.2% versus dorzolamide 2% each given three times daily to reduce intraocular pressure. Am J Ophthalmol 129:723–727
Stjernschantz J, Selen G, Ocklind A, Resul B (1998) Effects of latanoprost and related prostaglandin analogues. In: Alm A, Kaufman PL, Kitazawa Y, Lutjen-Drecoll E, Stjernschantz J, Weinreb RN (eds) Uveoscleral outflow: biology and clinical aspects. Mosby-Wolfe, London, pp 57–72
Strahlman E, Tipping R, Vogel R (1995) A double-masked, randomized 1-year study comparing dorzolamide (Trusopt), timolol, and betaxolol (International Dorzolamide Study Group). Arch Ophthalmol 113:1009–1016
Sugrue MF (2000) Pharmacological and ocular hypotensive properties of topical carbonic anhydrase inhibitors. Prog Retin Eye Res 19:87–112
Supuran CT, Scozzafava A (2000) Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opin Ther Pat 10:575–600
Sverrisson T, Gross R, Pearson J et al (1999) The dorzolamide/timolol combination versus timolol plus pilocarpine: patient preference and impact on daily life. United States Patient Preference Study Group. International Patient Preference Study Group. J Glaucoma 8:315–324
Tamm S, Tamm E, Rohen JW (1992) Age-related changes of the human ciliary muscle. A quantitative morphometric study. Mech Ageing Dev 62:209–221
The Advanced Glaucoma Intervention Study (AGIS) (2000) 7. The relationship between control of intraocular pressure and visual field deterioration. The AGIS Investigators. Am J Ophthalmol 130:429–440
The Levobunolol Study Group (1985) A beta-adrenoceptor antagonist effective in the long-term treatment of glaucoma. Ophthalmology 92:1271–1276
Thoft RA (1968) Incidence of lens changes in patients treated with echothiophate iodide. Arch Ophthalmol 80:317–320

Toris CB, Camras CB, Yablonski ME (1993) Effects of PhXA41, a new prostaglandin F2 alpha analog, on aqueous humor dynamics in human eyes. Ophthalmology 100:1297–1304
Toris CB, Yablonski ME, Wang YL, Camras CB (1999) Aqueous humor dynamics in the aging human eye. Am J Ophthalmol 127:407–412
Van Buskirk EM (1977) Trabeculotomy in the immature, enucleated human eye. Invest Ophthalmol Vis Sci 16:63–66
Van Buskirk EM (1980) Adverse reactions from timolol administration. Ophthalmology 87:447–450
van der Valk R, Webers CA, Schouten JS, Zeegers M, Hendrikse F, Prins M (2005) Intraocular pressure-lowering effects of all commonly used glaucoma drugs: a meta-analysis of randomized clinical trials. Ophthalmology 112:1177–1185
Vasudevan SK, Gupta V, Crowston JG (2011) Neuroprotection in glaucoma. Indian J Ophthalmol 59(Suppl):S102–S113
Velde TM, Kaiser FE (1983) Ophthalmic timolol treatment causing altered hypoglycemic response in a diabetic patient. Arch Intern Med 143:1627
Walters TR (1996) Development and use of brimonidine in treating acute and chronic elevations of intraocular pressure: a review of safety, efficacy, dose response, and dosing studies. Surv Ophthalmol 41:S19–S26
Wang SK, Chang RT (2014) An emerging treatment option for glaucoma: Rho kinase inhibitors. Clin Ophthalmol 8:883–890
Watson P, Stjernschantz J (1996) A six-month, randomized, double-masked study comparing latanoprost with timolol in open-angle glaucoma and ocular hypertension. The Latanoprost Study Group. Ophthalmology 103:126–137
Weinreb RN, Lindsey JD, Luo XX, Wang TH (1994) Extracellular matrix of the human ciliary muscle. J Glaucoma 3:70–78
Weinreb RN, Kashiwagi K, Kashiwagi F, Tsukahara S, Lindsey JD (1997) Prostaglandins increase matrix metalloproteinase release from human ciliary smooth muscle cells. Invest Ophthalmol Vis Sci 38:2772–2780
Weinreb RN, Ong T, Scassellati Sforzolini B, Vittitow JL, Singh K, Kaufman PL (2014) A randomised, controlled comparison of latanoprostene bunod and latanoprost 0.005% in the treatment of ocular hypertension and open angle glaucoma: the VOYAGER study. Br J Ophthalmol 96:738–745
Whitson JT, Henry C, Hughes B et al (2004) Comparison of the safety and efficacy of dorzolamide 2% and brimonidine 0.2% in patients with glaucoma or ocular hypertension. J Glaucoma 13:168–173
Wilson RP, Kanal N, Spaeth GL (1979) Timolol: its effectiveness in different types of glaucoma. Ophthalmology 86:43–50
WoldeMussie E, Ruiz G, Wijono M, Wheeler LA (2001) Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. Invest Ophthalmol Vis Sci 42:2849–2855
Zimmerman TJ (1997) Textbook of ocular pharmacology. Lippincott-Raven, Philadelphia, p 250
Zimmerman TJ, Kaufman HE (1977) Timolol: a beta-adrenergic blocking agent for the treatment of glaucoma. Arch Ophthalmol 95:601–604
Zimmerman TJ, Sharir M, Nardin GF et al (1992) Therapeutic index of pilocarpine, carbachol, and timolol with nasolacrimal occlusion. Am J Ophthalmol 114:1–7

Translational Pharmacology in Glaucoma Neuroprotection

Leonard A. Levin

Contents

L.A. Levin (✉)
Department of Ophthalmology, McGill University, Montreal, QC, Canada

Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

McGill Academic Eye Centre, 5252 de Maisonneuve West, Suite 400, Montreal, QC, Canada, H4A 3S5

Department of Ophthalmology, University of Montreal, Montreal, QC, Canada

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_20

Abstract

Glaucoma is both the most common optic neuropathy worldwide and the most common cause of irreversible blindness in the world. The only proven treatment for glaucomatous optic neuropathy is lowering the intraocular pressure, achieved with a variety of pharmacological, laser, and surgical approaches. Over the past 2 decades there has been much basic and clinical research into achieving treatment of the underlying optic nerve damage with neuroprotective approaches. However, none has resulted in regulatory approval based on successful phase 3 studies. This chapter discusses the reasons for this "lost in translation" aspect of glaucoma neuroprotection, and outlines issues at the laboratory and clinical trial level that need to be addressed for successful development of neuroprotective therapies.

Keywords

Glaucoma • Optic neuropathy • Retinal ganglion cells • Translational research

1 Introduction

Glaucomatous optic neuropathy is the most common cause of irreversible blindness in the world, and the second most common in developed countries such as North America and Europe. As discussed in the previous chapter on intraocular pressure lowering in glaucoma, glaucoma is a disease of the optic nerve. The optic nerve is a structure that contains approximately 1.2 million axons (nerve fibers) connecting retinal ganglion cell bodies in the inner retina to targets in the brain. The main target of the retinal ganglion cell axons is the lateral geniculate nucleus, but they also connect to the superior colliculus, pretectal nuclei, and suprachiasmatic nucleus of the hypothalamus.

Through processes that are incompletely understood, but probably involve biomechanical forces, there is damage to the axons at the level of the optic disc. The optic disc is the structure through which retinal ganglion cell axons make a 90° turn from the retina towards the optic nerve. It is unclear whether the damage to axons is direct, e.g., from stretch, transection, or ischemia, or indirect, e.g., from other cell types such as astrocytes and inflammatory cells.

Whatever the mechanism, the injury at the site of the optic disc in glaucoma results in a progressive loss of axons and cell bodies over time, disconnecting the retina and its upstream visual processing retinal neurons (photoreceptors, bipolar cells, horizontal cells, and amacrine cells) from the targets in the brain. There is evidence that there are upstream changes in afferent neurons in the retina in glaucoma (Choi et al. 2011; Werner et al. 2011), as well as downstream structural and functional changes in the target neurons in the lateral geniculate nucleus (Yucel et al. 2001; Gupta et al. 2009; Zhang et al. 2016) and secondary target visual cortex neurons responsible for visual processing (Crawford et al. 2001; Dekeyster et al. 2015; Borges et al. 2015). Nonetheless, most of the changes that are relevant

to vision loss in glaucoma reflect the structural loss of the retinal ganglion cell body and axon (Danesh-Meyer and Levin 2015).

It is not clear to what degree retinal ganglion cell functionality is lost before irreversible structural failure. In other words, the time interval between structural damage to the retinal ganglion cell and its loss of functionality is unclear in human glaucoma. This should be contrasted with diseases such as optic neuritis, where there can be conduction block of axonal transmission as a result of inflammatory-mediated demyelination. Such functional loss can be very transient, as in Uhthoff phenomenon in multiple sclerosis, where a rise in body temperature or exercise can cause a few minutes of visual loss, or somewhat more persistent, e.g., after demyelination in an optic neuritis episode, but with virtually complete functional recovery. In these cases, structural loss of retinal ganglion cell axons still occurs, but does not parallel the changes in function.

In contrast, the visual loss in glaucoma is predominantly one of structural failures of the retinal ganglion cell and its axon as a result of injury, and which is typically irreversible at both the functional and structural level. As discussed in the previous chapter, intraocular pressure lowering is efficacious in slowing the progressive loss of vision, e.g., as measured by visual field measurements. Lowering intraocular pressure is the only approved therapy for treating glaucoma, and is universally used.

2 Rationale for Neuroprotection

Given that retinal ganglion cell degeneration is a critical element for the irreversible loss of vision in glaucoma, it is long been considered worthwhile to test whether decreasing the degenerative process of this neuron could be useful as a therapy for glaucoma itself. Unlike other cells in the body, which can regenerate after injury, the retinal ganglion cell does not do so in higher animals, similar to other neurons. If the retinal ganglion cell could be kept alive and maintained in a functional state, then a neuroprotective therapy would be additive to therapies that lower intraocular pressure (Weinreb and Levin 1999).

3 Neuroprotection, Neuroenhancement, and Neuroregeneration

There are three aspects to improving function of retinal ganglion cells (Chang and Goldberg 2012). *Neuroprotection* is a therapy which prevents the degeneration of the retinal ganglion cells, and necessarily maintains its structural and functional capabilities. This is discussed at length later in this chapter. *Neuroenhancement* is improving function irrespective of structural changes. In this case, a retinal ganglion cell that is structurally present but which performs suboptimally with respect to visual conduction can have its function improved with a treatment. This is one of the least-studied areas of retinal ganglion cell treatment in glaucoma, but has

prospects for the future in those situations where structural loss is not very advanced.

The third therapy for improving visual function is *neuroregeneration*. Neuroregeneration is the reversal of preexisting structural loss of the retinal ganglion cell. There are two aspects to this reversal. In the first case, a retinal ganglion cell that has an intact cell body, but the axon has degenerated, is regenerated. This requires that the axon extend its growth cone through the optic nerve, chiasm, and optic tract, and then make its way to the appropriate (retinotopic) target areas in the lateral geniculate nucleus. Neuroregeneration has been the subject of research for decades. Recent studies have shown that it is possible to achieve substantial amounts of regeneration by manipulation of transcription factors within the retinal ganglion cell. Issues with respect to pathfinding between the eye and the targets are more complex, and probably require a combination of gradients of chemotactic molecules and/or cell surface guidance molecules for the extending growth cone of the axon.

The second aspect to neuroregeneration for glaucoma is when the retinal ganglion cell body itself has been lost. Retinal ganglion cell bodies primarily die by apoptosis after axonal injury, and for structural regeneration to take place in this circumstance, they need to be repopulated. Here, the use of stem cells or inducible dedifferentiated other cells has been shown to result in cells that are phenotypically retinal ganglion cells. These still need to regenerate their axons, as discussed previously, and make their way to appropriate targets in the brain.

4 Neuronal Therapies and Visual Function in Glaucoma

What are the implications of these three different aspects to retinal ganglion cell function in glaucoma? First of all, it should be recognized that intraocular pressure lowering can be thought of as an indirectly neuroprotective therapy, in that the progressive structural loss of the retinal ganglion cell (manifested by loss of the axons contained within the retinal nerve fiber layer over time) can be decreased with adequate intraocular pressure lowering. Second, in approaching the design of clinical trials for glaucoma, a therapy that decreases the rate of progressive loss of vision over time could be either neuroprotective or neuroenhancing. It is possible to distinguish these two possibilities by looking at what happens after the therapy is terminated. If the therapy is stopped and the visual function returns to where it would have been without treatment, then it is more likely that the therapy is working via a neuroenhancement effect rather than a neuroprotective effect. If the function maintains its level, then the therapy is more likely neuroprotective. Another way of distinguishing neuroprotection from neuroenhancement is assessment of structural metrics associated with the retinal ganglion cell over time, e.g., the thickness of the retinal nerve fiber layer, which would be preserved with a neuroprotective therapy but not a neuroenhancement therapy. A method for distinguishing a neuroregenerative therapy is based on the recovery of lost fibers, such as in the retinal nerve fiber layer. In other words, if the retinal nerve

fiber layer thickness increases over time and is coupled with functional recovery, then this would be prima facie evidence for a successful neuroregenerative therapy.

5 Processes of Cellular Dysfunction and Death in Glaucoma

The previous section provides a framework for understanding response to different types of therapy for retinal ganglion cells. The past decades have provided an enormous variety of laboratory studies in which retinal ganglion cell death and dysfunction have been ameliorated in experimental models of optic nerve disease, including experimental glaucoma. This topic has been thoroughly reviewed (Almasieh et al. 2012; Danesh-Meyer 2011; Nickells et al. 2012), and the many mechanisms and approaches that have been studied in various laboratories are listed in those references. This section will synopsize some of the main approaches that have been studied most recently. For each approach, there are implications with respect to whether those neuroprotective therapies would be translatable to human glaucoma.

The basic model that will be used here is that there is first an injury which takes place at the level of the optic disc, affecting retinal ganglion cell axons directly or indirectly. Secondarily, there are downstream effects on five neuronal compartments: (1) retinal ganglion cell bodies, which primarily undergo apoptosis; (2) the proximal axons, via a process called retrograde axonal degeneration; (3) the distal axons, via a process called Wallerian degeneration; (4) the afferent neurons in the inner and outer retina; and (5) efferent neurons in the lateral geniculate nucleus and other targets.

Early studies focused on the first neuronal compartment, i.e., maintaining the health of the retinal ganglion cell body after axonal injury. Studies in the 1990s used neurotrophic factors such as brain-derived neurotrophic factor, which maintains retinal ganglion cell survival during development, to improve their survival after axotomy. For example, studies from several laboratories of Aguayo and his students showed that intravitreal injection or delivery of brain-derived neurotrophic factor decreased the rate of ganglion cell loss after optic nerve transection (Mey and Thanos 1993; Mansour-Robaey et al. 1994; Di Polo et al. 1998). Similar studies with other neurotrophic factors and small molecules in vitro confirmed that retinal ganglion cells could be kept alive with cocktails of various neurotrophic factors (Meyer-Franke et al. 1995).

Over the years, the pathways by which axonal injury causes retinal ganglion cell apoptosis have been dissected to a fine level of detail. There are three major approaches to blocking these pathways, and thereby improving retinal ganglion cell survival in axonal injury. The first is to block the process of apoptosis itself. For example, a knockout of the apoptotic protein Bax in retinal ganglion cells maintains their viability despite severe axonal injury or experimental glaucoma (Isenmann et al. 1999; Libby et al. 2005). Another example is the use of caspase inhibitors, either via drugs or antisense methods (Kermer et al. 1998, 2000; Chaudhary et al. 1999; McKinnon et al. 2002). A second approach is to go further upstream

and block induction of the death pathways, mediated by messengers such as superoxide induction (Kanamori et al. 2010a, b; Catrinescu et al. 2012), loss of Jun kinase signaling (Harder et al. 2011; Fernandes et al. 2012), activation of dual leucine zipper kinase signaling (Welsbie et al. 2013; Huntwork-Rodriguez et al. 2013), beta-amyloid aggregation (Tsuruma et al. 2010; Parsons et al. 2015), or other intracellular signals. The third approach is to enhance the survivability of the retinal ganglion cell in the presence of induction of cell death. Neurotrophins are an example of this approach, as are erythropoietin (Tsai et al. 2005; Zhong et al. 2007; Suhs et al. 2012) and activation of other cell surface receptor-mediated pathways that improve viability, such as alpha-2 agonists (Yoles et al. 1999; Wheeler and Woldemussie 2001) or adenosine agonists (Perígolo-Vicente et al. 2013; Lieven et al. 2014; Galvao et al. 2013; Madeira et al. 2015; Lu et al. 2015).

6 Protection from Axonal Degeneration

Although maintaining the health and functionality of the cell body has been a successful approach to neuroprotection in vitro and in some animal models of optic nerve injury, this is not enough for translation to glaucoma. The pathophysiological nature of the glaucomatous process is injury to the axon, at the level of the optic disc. Without an intact axon, the cell body is disconnected from its target in the brain. It is therefore insufficient to keep retinal ganglion cells alive without preserving their axon.

There are two aspects to preserving axonal health in glaucoma. The first is maintaining the connectivity of the axon. An axon that has been disrupted cannot reconstitute itself by bringing the two injured ends back together, and therefore the consequences of irreversible axonal damage are disconnection and loss of functionality. A treatment that could make axons more robust and less likely to become transected under situations of biomechanical deformation, ischemia, or inflammation would be a significant advance for this mode of cellular injury. Unfortunately, there is so far less experimental data to support this therapeutic mechanism for disrupted axons in glaucoma in culture or in experimental animals.

The second aspect of axonal degeneration relates to when there is injury to the axon that is not sufficient to cause the axon itself to separate, but which causes subsequent downstream retrograde or Wallerian axonal degeneration of the axon. There is much known about how axons degenerate because of a remarkable naturally occurring animal model, the Wallerian degeneration slow (Wld^S) mouse. This animal expresses Wld^S, a fusion protein containing nicotinamide mononucleotide adenylyltransferase 1, which is important for NAD^+ synthesis. The Wld^S protein results in a slowed axonal degeneration in the face of traumatic or other injury. The mutation has been replicated in the rat, and both mice and rats have been used for experimental studies in glaucoma and other optic nerve injuries (Perry et al. 1991; Beirowski et al. 2008). The Wld^S mutation and other axon degeneration mutations have permitted exploration of the possibility that axonal

degeneration protection, or *axoprotection* (Levin and Peeples 2008; Ghaffarieh and Levin 2012), can be a therapy for glaucoma independent of cell body protection. Along the same lines, several drugs have been studied with respect to protection of the axon, separate from cell body protection (Kojima et al. 2012; Ma et al. 2012; Kitaoka et al. 2015; Sase et al. 2015; Ribas et al. 2016). As discussed in the section on animal models, it is possible to separate the survival of the cell body from survival of the axon in experimental models of optic nerve injury. Many drugs have been shown to maintain both axon survival and cell body survival, and presumably these are likely to be more translatable to human disease therapies than those that protect either the cell body or axon alone.

7 Structural Protection and Functional Protection

Separate from structural preservation is functional preservation. It is necessary that the entire neuron, i.e., the structure, be present for useful neuronal function to be possible. The converse is not true, however. Even with intact structure, there may be insufficient functionality of a retinal ganglion cell to maintain vision, and therefore therapies that maintain structure without maintaining function in animal models are less likely able to be translated to human disease. As discussed in the section on animal models, in recent years most researchers obtain a functional readout in addition to a structural readout in neuroprotection studies in glaucoma.

8 Importance of Site of Action

The need for preserving retinal ganglion cell bodies, their axons, and their connectivity was mentioned earlier. One of the concerns in drug development for glaucoma is that a drug may be highly effective in culture or in animal models for preventing cell death in one area, but when dosed in a human under physiological conditions may not achieve sufficient levels at the site necessary for neuroprotection. Note that the site of injury does not necessarily correspond to the site of action of a drug. For example, it is possible that a drug that has its primary effect on cell body preservation could be delivered to reach a sufficient concentration at the retina, even though the site of injury in the disease is at the optic disc. However, given the importance of maintaining the entire retinal ganglion cell, for most drugs this means that the site of action should include both the axonal and cell body compartments. In animals with small eyes such as rats and mice, a topical drug will achieve high levels both in the retina and in the anterior optic nerve, including the disc. However, in a human, topical drug application may reach much lower levels within the retina, and even lower levels at the optic disc and anterior optic nerve. This could lead to problems translating positive findings from the laboratory to the clinic. Potential solutions are drugs that achieve high systemic levels or periocular levels, those that affect gene transcription and thereby have effects throughout the entire retinal ganglion cell, or the use of novel delivery systems.

9 Historical Summary of Neuroprotection in Glaucoma

Starting in the 1990s, the concept of neuroprotection as an additional or alternative therapy to intraocular pressure lowering has captured excitement in those developing new therapies for this blinding disease. There have been several therapies that were studied in a small number of subjects, based primarily on the use of drugs that were already being used for other purposes, including intraocular pressure lowering. These included betaxolol (Vainio-Jylha and Vuori 1999; Araie et al. 2003), a beta-1 adrenergic antagonist, an L-type calcium channel blocker (Koseki et al. 2008), and brimonidine (Evans et al. 2003; Aung et al. 2004; Wilhelm et al. 2006; Newman et al. 2006), an alpha-2 adrenergic agonist. The latter was associated with apparent protection in patients with glaucoma where the intraocular pressure was measured within the normal range, the so-called normal-tension glaucoma (Krupin et al. 2011). This Low-pressure Glaucoma Treatment Study (LoGTS) demonstrated a lower rate of progression of visual field loss in subjects treated with brimonidine compared to timolol, a beta-adrenergic antagonist which is not neuroprotective, but which lowered intraocular pressure in this study to a similar extent as brimonidine. This small study was critiqued for differential dropout, changes in the statistical analysis, and other issues (Cordeiro and Levin 2011; Sena and Lindsley 2013).

The most well-known study of neuroprotection in glaucoma consisted of two clinical parallel trials of the oral *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine. These trials were sponsored by the pharmaceutical company Allergan, and involved more than 2,000 patients over up to 5 years. The results of these studies were never published, but press releases from the manufacturer disclosed that both clinical trials failed to meet their respective primary endpoint measures, which were based on visual function. Because of the large number of subjects in these studies and the failure to show efficacy, there was a feeling in the community that pursuing a neuroprotection indication in glaucoma was high-risk. Subsequent large-scale studies of memantine for this indication have not occurred, and it is only recently that other companies have started drug development in the neuroprotection space. A more detailed history of early neuroprotection development in glaucoma can be found elsewhere (Levin and Peeples 2008).

10 Translational Issues in Glaucoma Neuroprotection

Preclinical research does not always predict the results of clinical trials. This issue, which we have named the "Lost in Translation" problem (Levin and Danesh-Meyer 2010), can reflect either problems with the data from the preclinical studies, problems with the design of the clinical studies, or the biological failure of an animal model to predict what occurs in human subjects. Some of these issues can be managed (Ergorul and Levin 2013). This section will discuss some of the relevant issues associated with performing translational research in the development of neuroprotective drugs for glaucoma.

10.1 Screening Techniques In Vitro for Glaucoma Neuroprotection

Laboratories typically use the survival of retinal ganglion cells in culture as a rapid method for screening drugs for their neuroprotective efficacy. There are two common methods for retinal ganglion cell cultures. The first is to use a culture of mixed retinal cells, where the retinal ganglion cells have previously been fluorescently labeled by injection of a dye into the superior colliculus, the main target area of retinal ganglion cell axons in the rodent (Leifer et al. 1984). After sufficient time for the dye to be retrogradely transported from the superior colliculus to retinal ganglion cells within the retina, the retinas are removed, enzymatically dissociated, and the cells cultured. The effect of a drug on retinal ganglion cell survival can be assessed by counting the viability of those cells that have the fluorescent label within them, and which are therefore retinal ganglion cells. The second approach is to culture purified retinal ganglion cells. In this method, cells from enzymatically dissociated retinas are immunopurified with antibodies specific to retinal ganglion cells via cell surface Thy-1 (a retinal ganglion cell-specific marker), and then cultured and assessed using similar methods as for mixed retinal cultures (Barres et al. 1988). Note that attempts to find cell lines that have retinal ganglion cell characteristics have been unsuccessful (Krishnamoorthy et al. 2013).

In all of these cases, the main outcome is the viability of the cell body. However, the mechanism for injury in glaucoma is damage to the axon, with cell body death occurring secondarily. Therefore, although a drug may be neuroprotective in a screen where cell body viability is assessed, it would not necessarily be protective of the axon in a secondary screen. In addition, the mechanism of injury in these in vitro screen models is rarely axon injury. The very act of culturing the cells causes an axonal injury, because when the eye is removed, the optic nerve is cut, and when the retina is dissociated, retinal ganglion cells are disconnected from their axons. Therefore, the baseline cell culture conditions needed to maintain viability are simultaneously preventing death from axonal injury itself. In other words, the use of cell culture approaches for screening has a critical disadvantage. Nonetheless, there are no good alternatives.

A recent approach is to use long-term culture of purified retinal ganglion cells and allow the axon to reappear. The axon is then injured mechanically or with a femtosecond laser, and the effects on the axon and cell body are studied (Kunik et al. 2011). A related approach would be to assess the regeneration of an axon in the culture itself over time as a marker of a drug's ability to maintain axonal health.

The key endpoint in cell culture models is structural preservation of either the cell body, the axon, or both. Usually the dendritic tree is not studied, nor are the connections between the retinal ganglion cell and other efferent or afferent neurons. Function is rarely studied in screening procedures because electrophysiological testing is difficult to do as a high-throughput procedure. In general, dendrites, connectivity, and function are better studied in animal models.

10.2 Screening Techniques In Vivo for Glaucoma Neuroprotection

Animal studies of glaucoma use methods to elevate intraocular pressure. Although approximately 40–90% of patients with glaucoma can have intraocular pressure measurements in the range that is considered normal in 95% of the population, randomized studies have shown that lowering the intraocular pressure in those patients preserves vision over time as well as in patients with statistically higher pressure (Heijl et al. 2002; Leske et al. 2003). These findings are the bases for the primacy of elevated intraocular pressure in animal models. Studies using ischemia or inflammation in animal models have been performed, but their relevance and predictive capacity for human glaucoma are uncertain.

The subject of the different animal models available in glaucoma has been recently reviewed in a special issue of Experimental Eye Research (Ethier et al. 2015), and only some critical issues will be discussed here. Most laboratories use rodent models of elevated intraocular pressure (ocular hypertension) as their initial stage for testing drugs that were previously studied in vitro. There are two types of ocular hypertension models, inducible and genetic. In inducible models, the outflow of aqueous humor from the eye is reduced, raising the intraocular pressure. This can be done using hypertonic saline to sclerose the aqueous veins that are the outflow of the trabecular meshwork, the main filtering pathway in the eye. A more recent development is the injection of small particles such as plastic microbeads into the anterior chamber, which travel to the trabecular meshwork and obstruct it. The hypertonic saline model has been thoroughly assessed in the rat, while the microbead models have been most commonly assessed in the mouse.

The most common genetic model in rodents is the DBA/2 mouse, which develops a pigmentary-type glaucoma over time (John et al. 1998). The advantage of this genetic model is that breeding of these animals with other animals that have genetic manipulations allows dissection of critical pathways for cell death and dysfunction in glaucoma. The disadvantage of the DBA/2 and other genetic models is the substantial variability in the time to onset of the disease and the amount of neuronal loss in the glaucomatous process. This variability increases the number of animals per group.

Both the inducible and genetic models have great advantages in the rodent. Other lower animals develop glaucoma, either as an inducible model or based on species-specific risk of glaucoma, but are not used for screening procedures in drug development. Examples include pigs (Ruiz-Ederra et al. 2005), cats (McLellan and Miller 2011; Rutz-Mendicino et al. 2011), and dogs (Kuchtey et al. 2013).

10.3 Nonhuman Primates in Neuroprotection Drug Development

The nonhuman primate is the animal model that best mimics human glaucoma. The experimental method is to use an argon laser to thermally scar the trabecular meshwork, thereby blocking outflow and raising the intraocular pressure. The monkey eye, and specifically retina, is anatomically and physiologically very similar to the human eye. It is therefore likely that drugs which decrease the loss

of retinal ganglion cells and their function over time in this model will mimic that which occurs in the human. The main disadvantage of the nonhuman primate glaucoma model for drug development is that its significant variability in the response to the laser injury, and consequently the level of intraocular pressure in an individual animal. In addition, there is variability in the amount of retinal ganglion cell loss associated with a given level of intraocular pressure increase. Finally, it is difficult to do experiments on a large number of nonhuman primates because of their complexity of care, including need for adequate living space, provision of opportunities for social interactions, and consequent expense.

The above issues associated with using nonhuman primates for glaucoma neuroprotection result in a counterintuitive result in terms of the drug development process. If a drug is found to be neuroprotective in a rodent animal model, there may be circumstances where it makes more sense to go directly to a clinical proof-of-concept study instead of first performing a nonhuman primate proof-of-concept study. The early clinical study is preferred when the substantial variance in monkey outcomes coupled with the difficulty in carrying out large-scale monkey studies means that the power of the study to detect a significant difference is low. If a study is performed in nonhuman primates and has a negative result, then an otherwise potentially successful program could be halted in its tracks because of a false-negative outcome in an underpowered study. If, instead, an adequately powered clinical proof-of-concept study is the initial step, then the opportunity cost associated with erroneous reliance on a nonhuman primate study can be avoided.

The other difficulty with using small number of animals is the potential for a false-positive response. For example, analysis of the preclinical data supporting the memantine in glaucoma study demonstrated that the data to support the drug in the monkey was based on only eight animals for the functional outcome (Hare et al. 2004a), and was based on a post hoc analysis that looked at retinal ganglion cell survival in only the inferior half of the retina for the structural outcome (Hare et al. 2004b).

10.4 Neuroprotection Clinical Trials

Regulatory agencies such as FDA have stated that a functional primary outcome is currently necessary for a neuroprotective indication in glaucoma. Joint National Eye Institute (NEI) and FDA meetings (Csaky et al. 2008; Weinreb and Kaufman 2011) have resulted in constructive discussions on appropriate endpoints among clinical researchers, industry, academicians, the NEI, and the FDA. In general, the current US regulatory environment for labeling of a pharmaceutical agent as neuroprotective in glaucoma requires it to have an effect on reducing *functional* progression, i.e., a measure of vision that is clinically meaningful to the patient. Unlike diseases such as neovascular (wet) macular degeneration, which affects central vision early in the disease and therefore can have visual acuity as an endpoint, glaucoma affects the peripheral visual initially, and acuity is only lost

late in the disease. Therefore, functional measures for glaucoma usually rely on the visual field, for which FDA has publicly (Weinreb and Kaufman 2011) stated that:

> The progression of visual field loss will be suspected if five or more reproducible points, or visual field locations, have significant changes from baseline beyond the 5% probability levels for the glaucoma-change-probability (GCP) analysis. Alternatively, visual field progression is suspected if the between-group mean difference in threshold for the entire field is statistically and clinically significant. This is often at least 7 dB on more than one examination.

Structural measures such as optical coherence tomography could theoretically be acceptable to regulatory agencies once the correlation between those measures is highly correlated with function (Weinreb and Kaufman 2011), but for now, the effects of a drug on decreasing progression of visual field loss is the most practical approach to assessing neuroprotection in pivotal Phase 3 trials. Although clinical trials such as the memantine in glaucoma took years to perform and thousands of subjects, novel techniques in assessing the visual field, coupled with new methods for clinical trial design, are likely to lead to shorter durations and number of subjects in future studies (Quigley 2012; Garway-Heath et al. 2015).

There have been a few medium- and large-scale clinical trials in neuroprotection in glaucoma and related areas that have been published. Examples include the LoGTS study described above (Krupin et al. 2011), which compared topical brimonidine to timolol for patients with normal-tension glaucoma, the Brimonidine in Anterior Ischemic Optic Neuropathy (BRAION) study (Wilhelm et al. 2006), which assessed topical brimonidine vs. placebo for nonarteritic anterior ischemic optic neuropathy, a study assessing topic brimonidine in patients with monocular Leber hereditary optic neuropathy (Newman et al. 2005), and a study of intravenous erythropoietin in optic neuritis (Suhs et al. 2012). A more extensive listing of recent and ongoing trials of neuroprotection in related areas can be obtained from clinicaltrials.gov, a major repository for ongoing clinical trials and for which prior entry is required by most journals before a trial is published (DeAngelis et al. 2004). Table 1 contains a listing of several trials relevant to this chapter.

10.5 Strategies for Neuroprotection Drug Development for Glaucoma

In the past, the process of drug development for glaucoma was viewed as a pyramid (Levin 2003) (Fig. 1). At the base level were cell culture models of retinal ganglion cells and other neurons, and neuronal animal models in diseases unrelated to glaucoma. The second step was testing of the drug candidates from the base layer in an optic nerve injury model. The third step was testing the candidate in an experimental glaucoma model. Finally, the drug was tested in randomized controlled trials of glaucoma.

The problems with this metaphor are multiple. The potential for risk and failure is understated. The layers appear to rest firmly on one another, and each layer strongly supports the one above. There is no sense of translational failure, in that the

Table 1 Listing of neuroprotection clinical trials relevant to glaucoma in clinical trials.gov

NCT number	Title	Recruitment	Condition	Phase	Enrollment	Completion date
NCT00466479	Brimonidine vs ALTP in progressing human glaucoma	Completed	Glaucoma	4	50	Oct-02
NCT00317577	Study of medical treatment of low-pressure (normal-tension) glaucoma	Completed	Glaucoma	2	160	May-04
NCT00476138	Effect of epigallocatechin-gallate on inner retinal function in ocular hypertension and early glaucoma	Recruiting	Glaucoma	1, 2	40	Jul-08
NCT00683501	Safety, efficacy, and pharmacokinetic profile of DNB-001 in subjects with elevated intraocular pressure	Completed	Glaucoma	1, 2	54	Oct-08
NCT00844389	Near to Infrared (NIR) light neuroprotection in glaucomatous optic neuropathy	Active, not recruiting	Glaucoma	0	40	Mar-10
NCT00771043	A proof-of-concept study to correlate retinal nerve fiber layer changes in patients with multiple sclerosis treated with natalizumab or interferon beta 1-a	Withdrawn	Optic neuritis	4	50	Jun-10
NCT00739154	Protective effect of phenytoin on glaucoma	Not yet recruiting	Glaucoma		200	Dec-10
NCT00693485	Safety and effects of brimonidine intravitreal implant in patients with glaucomatous optic neuropathy	Completed	Glaucoma	2	70	Aug-11
NCT01544192	Impact of oral versatile antioxidants on glaucoma progression	Completed	Glaucoma	3	60	Feb-12
NCT01073813	Neuroprotection and repair in optic neuritis	Terminated	Optic neuritis	2	6	Jan-13
NCT01064505	Safety study of a single IVT injection of QPI-1007 in chronic optic nerve atrophy and recent onset NAION patients	Completed	Nonarteritic anterior ischemic optic neuropathy	1	48	Apr-13

(continued)

Table 1 (continued)

NCT number	Title	Recruitment	Condition	Phase	Enrollment	Completion date
NCT01408472	NT-501 CNTF implant for glaucoma: safety, neuroprotection, and neuroenhancement	Completed	Glaucoma	1	11	Oct-14
NCT01451593	Neuroprotection with phenytoin in optic neuritis	Completed	Optic neuritis	2	92	Mar-15
NCT01838174	A Phase IV trial of neuroprotection with ACTH in acute optic neuritis	Recruiting	Optic neuritis	4	60	Apr-15
NCT01802489	Amiloride clinical trial in optic neuritis	Active, not recruiting	Optic neuritis	2	46	Nov-15
NCT01987167	Defining the functional and neuroprotective potential of ACTHAR in acute optic neuritis	Recruiting	Optic neuritis	0	25	Dec-16
NCT01338389	Influence of oral treatment with citicoline for the prevention of radiation optic neuropathy in patients treated for uveal melanomas with proton beam therapy	Recruiting	Radiation optic neuropathy		80	Dec-18
NCT01936129	Investigating the neuroprotective effect of Cop-1 (Copaxone) in acute primary angle closure	Recruiting	Angle-closure glaucoma	3	196	

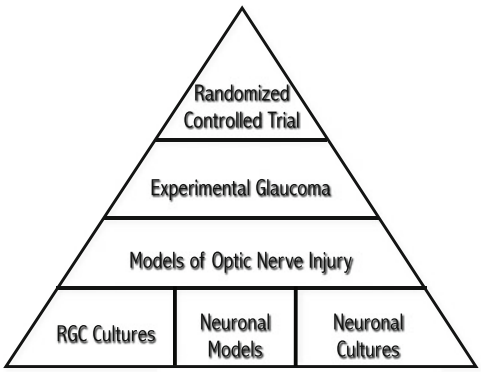

Fig. 1 An older view of drug development for neuroprotection in glaucoma, with a pyramid as a metaphor. The assumption is that drug development proceeds from the bottom to the top, with each level supporting the one above it. This is faulty (see text)

randomized controlled trial is the logical next step of a successful test in an animal glaucoma model. Finally, there is no consideration of a proof-of-concept study to support the initiation of a randomized controlled trial.

A better metaphor for neuroprotection drug development is like a game where a ball (the drug) is aimed at a target (Fig. 2). Here, the drug is brought to higher levels based on the application of preclinical studies and Phase 1 studies to support lack of toxicity. This part is relatively low risk. The height of the platform (i.e., the quality of the supporting data) determines the velocity of the ball down a chute, aimed at a second chute (representing the Phase 3 study). A second type of glaucoma model would increase the number of steps, and raise the height of the platform.

The likelihood that the ball will reach the second chute depends not just on the velocity of the ball, but also chance, because the second chute is much narrower than the first. The gap between the chutes and the narrowness of the second chute reflect the much smaller likelihood of success in Phase 3. Obtaining excellent preclinical data will help the ball reach the second chute and even make its way over the hump to success, but will not guarantee success because of the smaller width of the second chute. Finally, an intermediate platform with a spring represents a proof-of-concept trial, which can help the ball reach the second chute. The better the proof-of-concept study, the more likely the second chute will be reached.

11 Drug Delivery

The visual loss in glaucoma is slow and relentless, but usually asymptomatic until late in the course of the disease. This characteristic progression means that patients are unaware of their loss of vision until the visual field has constricted enough for them to realize that there is virtually no remaining peripheral vision. In some cases, the lack of awareness of vision loss will continue until central vision is involved and visual acuity decreases. Glaucoma is also usually painless, and unless there is a family member or friend who has severe visual loss from glaucoma, the patient may lack understanding of how devastating the visual loss can be.

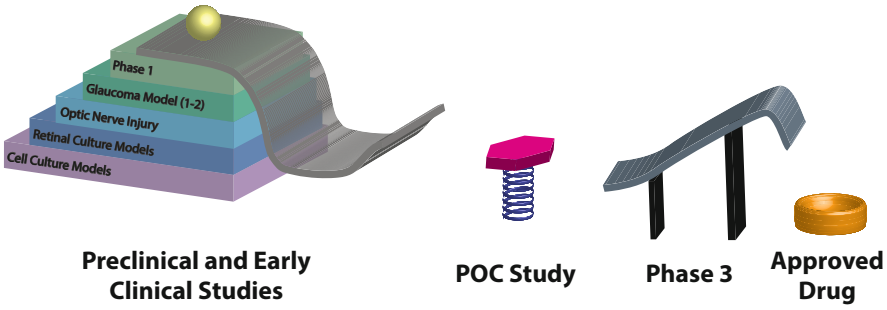

Fig. 2 A more accurate view of the challenges in neuroprotection drug development for glaucoma. The ball represents a drug, which is brought to the platform on the right by a series of preclinical and Phase 1 studies. The better the evidence in those studies, the higher the platform. The goal is for the drug to roll down the first chute, bridge the gap, hit the Phase 3 chute, and roll up high enough to go over the top (meet its endpoints) and arrive in the Approved Drug bowl. The narrow platform and the gap between the two chutes reflect the difficulties in translation from the laboratory to the clinic. A proof-of-concept study can help bridge the gap by serving as an intermediate aiming platform

Given that glaucoma is usually asymptomatic, a therapy for glaucoma should be something that is relatively uncomplicated and simple for patients. A drug or device that is painful, risky, or requires multiple physician visits may lead to diminished patient adherence. For example, a drug delivered by intravitreal injection every month may have lower adherence in glaucoma patients than one that can be applied topically via eye drops. Such a scenario is different from the therapies used for neovascular (wet) macular degeneration (AMD). Treatments for neovascular AMD are highly successful, and involved intravitreal injection of drugs that interfere with vascular endothelial growth factor (VEGF) (Lim et al. 2012). The visual loss in neovascular AMD is apparent early in the course of the disease, and each successive step of worse vision is often obvious to the patient. In addition, in neovascular macular degeneration treated with anti-VEGF drugs, the drug may improve vision over the short to medium term, thus making it even more obvious to the patient that the drug is both helpful and necessary. Improvement in glaucoma is rare.

Therefore, the choice of a drug delivery system is important in glaucoma neuroprotection. Most commonly, drugs that lower intraocular pressure are topically delivered as eye drops, and it is reasonable to attempt the same with a neuroprotection therapy. However, even eye drops can be difficult to deliver, especially in elderly patients. The eye can be missed when a drop is squeezed from a bottle held above the eye, particularly if there is a tremor or excessive blinking. Another approach is to deliver the drug orally, which has the advantage of treating both eyes simultaneously. However, the risk of adverse effects from systemic exposure with oral medications is usually worse. A third approach is a sustained-release formulation, which can be implanted around or in the eye, and replaced less frequently. For example, a drug that is given intravitreally and can provide constant levels of neuroprotection over the course of 3–6 months might have a much greater adherence in patients than one that needs to be administered

once every month. Such an approach also has the advantage that a patient is unable to forget to take their drug because it is a sustained-release formulation already being delivered.

12 The Rule of Three

Many disease treatments are only partly successful as monotherapies. However, when combined with other therapies, the result is often virtual eradication of a disease. For example, preventing conversion of human immunodeficiency virus infection to acquired immunodeficiency syndrome (AIDS) was only partly successful with the first major nucleoside analog reverse-transcriptase inhibitor, zidovudine (azidothymidine), which resulted in a 64% decrease in the development of AIDS (Volberding et al. 1990). Subsequent addition of didanosine further decreased conversion by 36% (Hammer et al. 1996). The use of triple therapy by adding a protease inhibitor such as indinavir (Hammer et al. 1997), and later, drugs like darunavir, decreased the development of AIDS by a further 50%, essentially allowing long-term survival in most patients with HIV infection. In other words, triple therapy results in a virtual eradication of the risk of AIDS. Another example is with coronary artery disease, where combining therapies that prevent or decrease formation of atherosclerotic plaque, improve cardiac function, and revascularize the heart are highly successful for improving quality of life and decreasing mortality. The third example is in treatment of tuberculosis infection, where 2–4 different antibiotics can be used in combination to treat the disease effectively.

In glaucoma, the mainstays of therapy are either to decrease aqueous production with drugs such as beta-adrenergic antagonists or carbonic anhydrase inhibitors, or increase aqueous outflow, either through the trabecular meshwork or the uveoscleral outflow pathways. The use of one, two, or in some cases three drugs, sometimes coupled with laser or incisional surgery to improve aqueous fluid dynamics, is highly successful in treating glaucoma. But as mentioned previously, even a combination of these treatments does not eradicate the disease. The addition of a third approach, i.e., neuroprotection, could theoretically make a dramatic difference in the outcomes of patients with glaucoma, and theoretically slowing progression to a rate that is essentially negligible. Although this cannot be predicted with certainty, there is potential for the "rule of three" approach to glaucoma (decreasing inflow, increasing outflow, and neuroprotection) to cure the disease.

13 Summary

The history of neuroprotection in glaucoma includes seminal studies in the laboratory delineating mechanisms of retinal ganglion cell death after axonal injury that are amenable to treatment, but also difficulties in translating these to the clinic. There are multiple pathways for retinal ganglion cell death that have been described, many of which can be targeted with drug treatment. However, the need to maintain the structure, connectivity, and function of both the cell body

and the axon means that in vitro experiments are insufficient for preclinical proof-of-concept, and animal models are required. These then need to be translated into clinical trials with function as a primary outcome, which can be done in less time and with fewer subjects than were used historically.

References

Almasieh M, Wilson AM, Morquette B, Cueva Vargas JL, Di Polo A (2012) The molecular basis of retinal ganglion cell death in glaucoma. Prog Retin Eye Res 31(2):152–181. doi:10.1016/j.preteyeres.2011.11.002

Araie M, Azuma I, Kitazawa Y (2003) Influence of topical betaxolol and timolol on visual field in Japanese open-angle glaucoma patients. Jpn J Ophthalmol 47(2):199–207

Aung T, Oen FT, Wong HT, Chan YH, Khoo BK, Liu YP, Ho CL, See J, Thean LH, Viswanathan AC, Seah SK, Chew PT (2004) Randomised controlled trial comparing the effect of brimonidine and timolol on visual field loss after acute primary angle closure. Br J Ophthalmol 88(1):88–94

Barres BA, Silverstein BE, Corey DP, Chun LL (1988) Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning. Neuron 1 (9):791–803

Beirowski B, Babetto E, Coleman MP, Martin KR (2008) The WldS gene delays axonal but not somatic degeneration in a rat glaucoma model. Eur J Neurosci 28(6):1166–1179

Borges VM, Danesh-Meyer HV, Black JM, Thompson B (2015) Functional effects of unilateral open-angle glaucoma on the primary and extrastriate visual cortex. J Vis 15(15):9–9

Catrinescu MM, Chan W, Mahammed A, Gross Z, Levin LA (2012) Superoxide signaling and cell death in retinal ganglion cell axotomy: Effects of metallocorroles. Exp Eye Res 97(1):31–35. doi:10.1016/j.exer.2012.02.006

Chang EE, Goldberg JL (2012) Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement. Ophthalmology 119(5):979–986

Chaudhary P, Ahmed F, Quebada P, Sharma SC (1999) Caspase inhibitors block the retinal ganglion cell death following optic nerve transection. Mol Brain Res 67(1):36–45

Choi SS, Zawadzki RJ, Lim MC, Brandt JD, Keltner JL, Doble N, Werner JS (2011) Evidence of outer retinal changes in glaucoma patients as revealed by ultrahigh-resolution in vivo retinal imaging. Br J Ophthalmol 95(1):131–141. doi:10.1136/bjo.2010.183756

Cordeiro MF, Levin LA (2011) Clinical evidence for neuroprotection in glaucoma. Am J Ophthalmol 152(5):715–716. doi:10.1016/j.ajo.2011.06.015

Crawford ML, Harwerth RS, Smith EL 3rd, Mills S, Ewing B (2001) Experimental glaucoma in primates: changes in cytochrome oxidase blobs in V1 cortex. Invest Ophthalmol Vis Sci 42 (2):358–364

Csaky KG, Richman EA, Ferris FL 3rd (2008) Report from the NEI/FDA Ophthalmic Clinical Trial Design and Endpoints Symposium. Invest Ophthalmol Vis Sci 49(2):479–489

Danesh-Meyer HV (2011) Neuroprotection in glaucoma: recent and future directions. Curr Opin Ophthalmol 22(2):78–86. doi:10.1097/ICU.0b013e32834372ec

Danesh-Meyer HV, Levin LA (2015) Glaucoma as a neurodegenerative disease. J Neuroophthalmol 35:S22–S28

DeAngelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, Kotzin S, Laine C, Marusic A, Overbeke AJ, Schroeder TV, Sox HC, Van Der Weyden MB (2004) Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med 351 (12):1250–1251

Dekeyster E, Aerts J, Valiente-Soriano FJ, De Groef L, Vreysen S, Salinas-Navarro M, Vidal-Sanz M, Arckens L, Moons L (2015) Ocular hypertension results in retinotopic alterations in the visual cortex of adult mice. Curr Eye Res 40(12):1269–1283

Di Polo A, Aigner LJ, Dunn RJ, Bray GM, Aguayo AJ (1998) Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells. Proc Natl Acad Sci U S A 95(7):3978–3983

Ergorul C, Levin LA (2013) Solving the lost in translation problem: improving the effectiveness of translational research. Curr Opin Pharmacol 13(1):108–114. doi:10.1016/j.coph.2012.08.005

Ethier CR, Morrison JC, Clark AF (2015) Introduction to special issue on glaucomatous optic neuropathy: In vivo models and techniques. Exp Eye Res 141:1–2

Evans DW, Hosking SL, Gherghel D, Bartlett JD (2003) Contrast sensitivity improves after brimonidine therapy in primary open angle glaucoma: a case for neuroprotection. Br J Ophthalmol 87(12):1463–1465

Fernandes KA, Harder JM, Fornarola LB, Freeman RS, Clark AF, Pang IH, John SW, Libby RT (2012) JNK2 and JNK3 are major regulators of axonal injury-induced retinal ganglion cell death. Neurobiol Dis 46(2):393–401. doi:10.1016/j.nbd.2012.02.003

Galvao J, Guo L, Santiago AR, Ambrosio A, Cordeiro MF (2013) Adenosine A3 receptor agonist inhibits retinal ganglion cell apoptosis in vivo. Invest Ophthalmol Vis Sci 54(15):433–433

Garway-Heath DF, Zhu H, Crabb DP (2015) Imaging improves the accuracy of visual field progression analysis in glaucoma: structure-guided ANSWERS. Invest Ophthalmol Vis Sci 56(7):2058–2058

Ghaffarieh A, Levin LA (2012) Optic nerve disease and axon pathophysiology. Int Rev Neurobiol 105:1–17. doi:10.1016/B978-0-12-398309-1.00002-0

Gupta N, Greenberg G, de Tilly LN, Gray B, Polemidiotis M, Yucel YH (2009) Atrophy of the lateral geniculate nucleus in human glaucoma detected by magnetic resonance imaging. Br J Ophthalmol 93(1):56–60. doi:10.1136/bjo.2008.138172

Hammer SM, Katzenstein DA, Hughes MD, Gundacker H, Schooley RT, Haubrich RH, Henry WK, Lederman MM, Phair JP, Niu M (1996) A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. N Engl J Med 335(15):1081–1090

Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, Eron JJ, Feinberg JE, Balfour HH, Deyton LR, Chodakewitz JA, Fischl MA, Phair JP, Pedneault L, Nguyen B-Y, Cook JC (1997) A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. N Engl J Med 337(11):725–733. doi:10.1056/NEJM199709113371101

Harder JM, Fernandes KA, Gan L, Libby RT (2011) JNK-dependent JUN signaling is critical to retinal ganglion cell death after axonal injury. Invest Ophthalmol Vis Sci 52(14):3076–3076

Hare WA, WoldeMussie E, Lai RK, Ton H, Ruiz G, Chun T, Wheeler L (2004a) Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, I: Functional measures. Invest Ophthalmol Vis Sci 45(8):2625–2639

Hare WA, WoldeMussie E, Weinreb RN, Ton H, Ruiz G, Wijono M, Feldmann B, Zangwill L, Wheeler L (2004b) Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, II: Structural measures. Invest Ophthalmol Vis Sci 45(8):2640–2651

Heijl A, Leske MC, Bengtsson B, Hyman L, Hussein M (2002) Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol 120(10):1268–1279

Huntwork-Rodriguez S, Wang B, Watkins T, Ghosh AS, Pozniak CD, Bustos D, Newton K, Kirkpatrick DS, Lewcock JW (2013) JNK-mediated phosphorylation of DLK suppresses its ubiquitination to promote neuronal apoptosis. J Cell Biol 202(5):747–763

Isenmann S, Engel S, Gillardon F, Bahr M (1999) Bax antisense oligonucleotides reduce axotomy-induced retinal ganglion cell death in vivo by reduction of Bax protein expression. Cell Death Differ 6(7):673–682

John SW, Smith RS, Savinova OV, Hawes NL, Chang B, Turnbull D, Davisson M, Roderick TH, Heckenlively JR (1998) Essential iris atrophy, pigment dispersion, and glaucoma in DBA/2J mice. Invest Ophthalmol Vis Sci 39(6):951–962

Kanamori A, Catrinescu MM, Kanamori N, Mears KA, Beaubien R, Levin LA (2010a) Superoxide is an associated signal for apoptosis in axonal injury. Brain 133(9):2612–2625

Kanamori A, Catrinescu MM, Mahammed A, Gross Z, Levin LA (2010b) Neuroprotection against superoxide anion radical by metallocorroles in cellular and murine models of optic neuropathy. J Neurochem 114(2):488–498

Kermer P, Klocker N, Labes M, Bahr M (1998) Inhibition of CPP32-like proteases rescues axotomized retinal ganglion cells from secondary cell death in vivo. J Neurosci 18 (12):4656–4662

Kermer P, Ankerhold R, Klocker N, Krajewski S, Reed JC, Bahr M (2000) Caspase-9: involvement in secondary death of axotomized rat retinal ganglion cells in vivo. Brain Res Mol Brain Res 85(1–2):144–150

Kitaoka Y, Kojima K, Munemasa Y, Sase K, Takagi H (2015) Axonal protection by brimonidine with modulation of p62 expression in TNF-induced optic nerve degeneration. Graefes Arch Clin Exp Ophthalmol 253:1291–1296

Kojima K, Kitaoka Y, Munemasa Y, Ueno S (2012) Axonal protection via modulation of the amyloidogenic pathway in tumor necrosis factor–induced optic neuropathyoptic nerve protection by γ-secretase inhibition. Invest Ophthalmol Vis Sci 53(12):7675–7683

Koseki N, Araie M, Tomidokoro A, Nagahara M, Hasegawa T, Tamaki Y, Yamamoto S (2008) A placebo-controlled 3-year study of a calcium blocker on visual field and ocular circulation in glaucoma with low-normal pressure. Ophthalmology 115(11):2049–2057

Krishnamoorthy RR, Clark AF, Daudt D, Vishwanatha JK, Yorio T (2013) A forensic path to RGC-5 cell line identification: lessons learned. Invest Ophthalmol Vis Sci 54(8):5712–5719

Krupin T, Liebmann JM, Greenfield DS, Ritch R, Gardiner S (2011) A randomized trial of brimonidine versus timolol in preserving visual function: results from the Low-Pressure Glaucoma Treatment Study. Am J Ophthalmol 151(4):671–681. doi:10.1016/j.ajo.2010.09.026

Kuchtey J, Kunkel J, Esson D, Sapienza JS, Ward DA, Plummer CE, Gelatt KN, Kuchtey RW (2013) Screening ADAMTS10 in dog populations supports Gly661Arg as the glaucoma-causing variant in beagles. Invest Ophthalmol Vis Sci 54(3):1881

Kunik D, Dion C, Ozaki T, Levin LA, Costantino S (2011) Laser-based single-axon transection for high-content axon injury and regeneration studies. PLoS One 6(11):e26832. doi:10.1371/journal.pone.0026832

Leifer D, Lipton SA, Barnstable CJ, et al (1984) Monoclonal antibody to Thy-1 enhances regeneration of processes by rat retinal ganglion cells in culture. Science 224:303–306

Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E (2003) Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. Arch Ophthalmol 121(1):48–56

Levin LA (2003) Retinal ganglion cells and neuroprotection for glaucoma. Surv Ophthalmol 48 (Suppl 1):S21–S24

Levin LA, Danesh-Meyer HV (2010) Lost in translation: Bumps in the road between bench and bedside. JAMA 303(15):1533–1534

Levin LA, Peeples P (2008) History of neuroprotection and rationale as a therapy for glaucoma. Am J Manag Care 14(Suppl 1):S11–S14

Libby RT, Li Y, Savinova OV, Barter J, Smith RS, Nickells RW, John SW (2005) Susceptibility to neurodegeneration in a glaucoma is modified by Bax gene dosage. PLoS Genet 1(1):17–26. doi:10.1371/journal.pgen.0010004

Lieven CJ, Levin LA, McVicar WK (2014) Evaluation of the neuroprotective effects of trabodenoson in a model of acute ocular hypertension. Invest Ophthalmol Vis Sci 55 (13):2427–2427

Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY (2012) Age-related macular degeneration. Lancet 379(9827):1728–1738

Lu W, Hu H, Sévigny J, B'Ann TG, Kaufman PL, Johnson EC, Morrison JC, Zode GS, Sheffield VC, Zhang X (2015) Rat, mouse, and primate models of chronic glaucoma show sustained elevation of extracellular ATP and altered purinergic signaling in the posterior eyeincreased extracellular ATP in chronic glaucoma. Invest Ophthalmol Vis Sci 56(5):3075–3083

Ma M, Shofer FS, Neumar RW (2012) Calpastatin overexpression protects axonal transport in an in vivo model of traumatic axonal injury. J Neurotrauma 29(16):2555–2563
Madeira MH, Elvas F, Boia R, Gonçalves FQ, Cunha RA, Ambrósio AF, Santiago AR (2015) Adenosine A2AR blockade prevents neuroinflammation-induced death of retinal ganglion cells caused by elevated pressure. J Neuroinflammation 12(1):115
Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A 91(5):1632–1636
McKinnon SJ, Lehman DM, Kerrigan-Baumrind LA, Merges CA, Pease ME, Kerrigan DF, Ransom NL, Tahzib NG, Reitsamer HA, Levkovitch-Verbin H, Quigley HA, Zack DJ (2002) Caspase activation and amyloid precursor protein cleavage in rat ocular hypertension. Invest Ophthalmol Vis Sci 43(4):1077–1087
McLellan GJ, Miller PE (2011) Feline glaucoma – a comprehensive review. Vet Ophthalmol 14 (Suppl 1):15–29. doi:10.1111/j.1463-5224.2011.00912.x
Mey J, Thanos S (1993) Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res 602(2):304–317
Meyer-Franke A, Kaplan MR, Pfrieger FW, Barres BA (1995) Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron 15:805–819
Newman NJ, Biousse V, David R, Bhatti MT, Hamilton SR, Farris BK, Lesser RL, Newman SA, Turbin RE, Chen K, Keaney RP (2005) Prophylaxis for second eye involvement in leber hereditary optic neuropathy: an open-labeled, nonrandomized multicenter trial of topical brimonidine purite. Am J Ophthalmol 140(3):407–415
Newman NJ, Biousse V, Newman SA, Bhatti MT, Hamilton SR, Farris BK, Lesser RL, Turbin RE (2006) Progression of visual field defects in leber hereditary optic neuropathy: experience of the LHON treatment trial. Am J Ophthalmol 141(6):1061–1067
Nickells RW, Howell GR, Soto I, John SW (2012) Under pressure: cellular and molecular responses during glaucoma, a common neurodegeneration with axonopathy. Annu Rev Neurosci 35:153–179. doi:10.1146/annurev.neuro.051508.135728
Parsons CG, Ruitenberg M, Freitag CE, Sroka-Saidi K, Russ H, Rammes G (2015) MRZ-99030–A novel modulator of Aβ aggregation: I–Mechanism of action (MoA) underlying the potential neuroprotective treatment of Alzheimer's disease, glaucoma and age-related macular degeneration (AMD). Neuropharmacology 92:158–169
Perígolo-Vicente R, Ritt K, Pereira MR, Torres PMM, Paes-de-Carvalho R, Giestal-de-Araujo E (2013) IL-6 treatment increases the survival of retinal ganglion cells in vitro: the role of adenosine A1 receptor. Biochem Biophys Res Commun 430(2):512–518
Perry VH, Brown MC, Lunn ER (1991) Very slow retrograde and Wallerian degeneration in the CNS of C57BL/Ola mice. Eur J Neurosci 3(1):102–105
Quigley HA (2012) Clinical trials for glaucoma neuroprotection are not impossible. Curr Opin Ophthalmol 23(2):144–154. doi:10.1097/ICU.0b013e32834ff490
Ribas VT, Koch JC, Michel U, Bähr M, Lingor P (2016) Attenuation of axonal degeneration by calcium channel inhibitors improves retinal ganglion cell survival and regeneration after optic nerve crush. Mol Neurobiol 1–15
Ruiz-Ederra J, Garcia M, Hernandez M, Urcola H, Hernandez-Barbachano E, Araiz J, Vecino E (2005) The pig eye as a novel model of glaucoma. Exp Eye Res 81(5):561–569. doi:10.1016/j.exer.2005.03.014
Rutz-Mendicino MM, Snella EM, Jens JK, Gandolfi B, Carlson SA, Kuehn MH, McLellan GJ, Ellinwood NM (2011) Removal of potentially confounding phenotypes from a Siamese-derived feline glaucoma breeding colony. Comp Med 61(3):251–257
Sase K, Kitaoka Y, Munemasa Y, Kojima K, Takagi H (2015) Axonal protection by short-term hyperglycemia with involvement of autophagy in TNF-induced optic nerve degeneration. Front Cell Neurosci 9:425

Sena DF, Lindsley K (2013) Neuroprotection for treatment of glaucoma in adults. Cochrane Database Syst Rev 2

Suhs KW, Hein K, Sattler MB, Gorlitz A, Ciupka C, Scholz K, Kasmann-Kellner B, Papanagiotou P, Schaffler N, Restemeyer C, Bittersohl D, Hassenstein A, Seitz B, Reith W, Fassbender K, Hilgers R, Heesen C, Bahr M, Diem R (2012) A randomized, double-blind, phase 2 study of erythropoietin in optic neuritis. Ann Neurol 72(2):199–210. doi:10.1002/ana.23573

Tsai JC, Wu L, Worgul B, Forbes M, Cao J (2005) Intravitreal administration of erythropoietin and preservation of retinal ganglion cells in an experimental rat model of glaucoma. Curr Eye Res 30(11):1025–1031

Tsuruma K, Tanaka Y, Shimazawa M, Hara H (2010) Induction of amyloid precursor protein by the neurotoxic peptide, amyloid-beta 25-35, causes retinal ganglion cell death. J Neurochem 113(6):1545–1554. doi:10.1111/j.1471-4159.2010.06724.x

Vainio-Jylha E, Vuori ML (1999) The favorable effect of topical betaxolol and timolol on glaucomatous visual fields: a 2-year follow-up study. Graefes Arch Clin Exp Ophthalmol 237(2):100–104

Volberding PA, Lagakos SW, Koch MA, Pettinelli C, Myers MW, Booth DK, Balfour HH, Reichman RC, Bartlett JA, Hirsch MS, Murphy RL, Hardy WD, Soeiro R, Fischl MA, Bartlett JG, Merigan TC, Hyslop NE, Richman DD, Valentine FT, Corey L (1990) Zidovudine in asymptomatic human immunodeficiency virus infection. N Engl J Med 322(14):941–949. doi:10.1056/NEJM199004053221401

Weinreb RN, Kaufman PL (2011) Glaucoma research community and FDA look to the future, II: NEI/FDA Glaucoma Clinical Trial Design and Endpoints Symposium: measures of structural change and visual function. Invest Ophthalmol Vis Sci 52(11):7842

Weinreb RN, Levin LA (1999) Is neuroprotection a viable therapy for glaucoma? Arch Ophthalmol 117(11):1540–1544

Welsbie DS, Yang Z, Ge Y, Mitchell KL, Zhou X, Martin SE, Berlinicke CA, Hackler L Jr, Fuller J, Fu J, Cao LH, Han B, Auld D, Xue T, Hirai S, Germain L, Simard-Bisson C, Blouin R, Nguyen JV, Davis CH, Enke RA, Boye SL, Merbs SL, Marsh-Armstrong N, Hauswirth WW, Diantonio A, Nickells RW, Inglese J, Hanes J, Yau KW, Quigley HA, Zack DJ (2013) Functional genomic screening identifies dual leucine zipper kinase as a key mediator of retinal ganglion cell death. Proc Natl Acad Sci U S A 110(10):4045–4050. doi:10.1073/pnas.1211284110

Werner J, Keltner J, Zawadzki R, Choi S (2011) Outer retinal abnormalities associated with inner retinal pathology in nonglaucomatous and glaucomatous optic neuropathies. Eye 25(3):279–289

Wheeler LA, Woldemussie E (2001) Alpha-2 adrenergic receptor agonists are neuroprotective in experimental models of glaucoma. Eur J Ophthalmol 11(Suppl 2):S30–S35

Wilhelm B, Ludtke H, Wilhelm H (2006) Efficacy and tolerability of 0.2% brimonidine tartrate for the treatment of acute non-arteritic anterior ischemic optic neuropathy (NAION): a 3-month, double-masked, randomised, placebo-controlled trial. Graefes Arch Clin Exp Ophthalmol 244(5):551–558

Yoles E, Wheeler LA, Schwartz M (1999) Alpha2-adrenoreceptor agonists are neuroprotective in a rat model of optic nerve degeneration. Invest Ophthalmol Vis Sci 40(1):65–73

Yucel YH, Zhang Q, Weinreb RN, Kaufman PL, Gupta N (2001) Atrophy of relay neurons in magno- and parvocellular layers in the lateral geniculate nucleus in experimental glaucoma. Invest Ophthalmol Vis Sci 42(13):3216–3222

Zhang P, Wen W, Sun X, He S (2016) Selective reduction of fMRI responses to transient achromatic stimuli in the magnocellular layers of the LGN and the superficial layer of the SC of early glaucoma patients. Hum Brain Mapp 37(2):558–569

Zhong L, Bradley J, Schubert W, Ahmed E, Adamis AP, Shima DT, Robinson GS, Ng YS (2007) Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. Invest Ophthalmol Vis Sci 48(3):1212–1218

Pharmacologic Treatment of Noninfectious Uveitis

Jared E. Knickelbein, Karen R. Armbrust, Meredith Kim, H. Nida Sen, and Robert B. Nussenblatt

Contents

Abstract

Uveitis encompasses a spectrum of diseases whose common feature is intraocular inflammation, which may be infectious or noninfectious in etiology (Nussenblatt and Whitcup 2010). Infectious causes of uveitis are typically

J.E. Knickelbein • K.R. Armbrust • M. Kim • H.N. Sen • R.B. Nussenblatt (✉)
Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bldg 10 Room 10N109, 10 Center Drive, Bethesda, MD 20892, USA
e-mail: DrBob@nei.nih.gov

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_21

treated with appropriate antimicrobial therapy and will not be discussed in this chapter. Noninfectious uveitides are thought have an autoimmune component to their etiology and are thus treated with anti-inflammatory agents.

Keywords
Antimetabolites • Biologic agents • Noninfectious uveitis • T cell inhibitors • Treatment

1 Introduction

Uveitis encompasses a spectrum of diseases whose common feature is intraocular inflammation, which may be infectious or noninfectious in etiology (Nussenblatt and Whitcup 2010). Infectious causes of uveitis are typically treated with appropriate antimicrobial therapy and will not be discussed in this chapter. Noninfectious uveitides are thought have an autoimmune component to their etiology and are thus treated with anti-inflammatory agents.

Uveitis may affect various sites within the eye, and the Standardization of Uveitis Nomenclature working group has recommended the following classifications: (1) Anterior uveitis in cases where the anterior chamber is the primary site of inflammation, (2) intermediate uveitis in cases where the vitreous is the primary site of inflammation, (3) posterior uveitis in cases where the retina or choroid are the primary sites of inflammation, and (4) panuveitis in cases where all of these sites are involved (Jabs et al. 2005). Depending on the primary sites of inflammation, different routes of anti-inflammatory therapy may be most appropriate. Below, we discuss the various anti-inflammatory agents and various routes of administration that may be employed in the pharmacologic treatment of noninfectious uveitis.

2 Corticosteroids

The first reports of corticosteroid use in the treatment of noninfectious uveitis were in the early 1950s (Woods 1950; Gordon et al. 1951). Corticosteroids remain a critical treatment modality for acute control of inflammation in noninfectious uveitis because of their rapid onset of action as well as their broad and robust anti-inflammatory effects. Corticosteroids function to activate anti-inflammatory genes while suppressing proinflammatory genes (Barnes 2011). Corticosteroids diffuse across the cell membrane, bind cytosolic glucocorticoid receptor (GR) alpha, and the corticosteroid/GR complex then translocates to the cell nucleus. In the nucleus, corticosteroid/GR complexes may act in several ways to promote anti-inflammatory and suppress proinflammatory responses. GR homodimers may bind glucocorticoid response elements (GREs) within promoter regions of corticosteroid-responsive genes, thereby activating anti-inflammatory

gene transcription in a process known as trans-activation. Rarely, GR homodimers interact with GREs to suppress proinflammatory gene transcription in a process known as cis-repression. Corticosteroid/GR complexes may also interact with and suppress the signaling pathways activated by proinflammatory transcription factors, such as nuclear factor-kB (NF-kB), thereby blocking gene transcription of proinflammatory genes in a process known as trans-repression. The downstream effects of these gene interactions include inhibition of cytokine, chemokine, and adhesion molecule expression, which in turn leads to reduced chemotaxis and function of immune cells. In addition, corticosteroids have been shown to upregulate proteins involved in tight junction formation between retinal endothelial cells, suggesting a role in stabilizing the blood–retinal barrier and potentially reducing influx of inflammatory cells and molecules into the normally immune-privileged intraocular environment (Felinski et al. 2008; Keil et al. 2013). The full range of molecular mechanisms employed by corticosteroids in suppressing inflammation has yet to be elucidated and remains an active area of investigation.

Given the large number of genes and proteins affected by corticosteroids, it is not surprising that corticosteroid treatment is accompanied by a variety of side effects, several of which are potentially very serious. Systemic side effects include increased risk of infection, Cushing's syndrome, osteoporosis, hypertension, dyslipidemia, adrenal insufficiency, insulin resistance, very rarely avascular necrosis of the joints, and growth retardation in children. Corticosteroid treatment also has specific ocular side effects, namely, development or progression of cataract and elevation of intraocular pressure (IOP) potentially leading to glaucoma, when administered locally in or around the eye. These adverse effects limit the long-term use of corticosteroids.

As mentioned above, corticosteroids may be administered systemically or locally to eye. Multiple formulations are available for both systemic and local ocular use (Table 1). In general, the location and severity of intraocular

Table 1 Corticosteroids commonly used in the treatment of noninfectious uveitis

Route of administration	Generic corticosteroid name	Available formulations (name, company)	Typical dosing in uveitis
Topical	Prednisolone acetate	Generic Pred Forte (Allergan)	Four times daily – every 1 h
	Difluprednate	Durezol (Alcon)	Four to eight times daily
Periocular	Triamcinolone	Kenalog (preserved; Bristol-Myers Squibb)	20–40 mg
Intravitreal	Triamcinolone	Triesence (non-preserved; Alcon)	2–4 mg
	Dexamethasone implant	Ozurdex (Allergan)	0.7 mg
	Fluocinolone acetonide implant	Retisert (Bausch & Lomb)	0.59 mg
Oral	Prednisone	Multiple including generic	1 mg/kg/day with taper
Intravenous	Methylprednisolone	Multiple including generic	1,000 mg/day for 3 days followed by oral taper

inflammation dictates the mode and specific corticosteroid used. The different corticosteroid formulations and routes of delivery are discussed in detail below.

2.1 Local Corticosteroids

Various options currently exist for local ocular corticosteroid administration, including topical formulations, preparations for injection in the sub-Tenon's space, inferior orbit, or vitreous cavity, as well as sustained release intraocular implants placed in the posterior segment. Topical formulations are primarily used for treatment for anterior uveitis, although they may also be utilized for mild vitreous inflammation and uveitic macular edema. The goal of topical corticosteroid treatment of anterior uveitis is to eliminate the cellular and inflammatory protein (flare) responses in the anterior chamber to prevent irreversible inflammatory damage to the eye in addition to reducing patient symptoms of ocular pain and light sensitivity. Unfortunately, long-term use and higher corticosteroid potency often result in complications of cataract formation or progression and elevation of intraocular pressure (Becker and Mills 1963; Urban and Cotlier 1986). Systemic side effects are rare but have been reported with topical corticosteroid treatment (Sendrowski et al. 2008).

Many topical corticosteroid formulations are available, and they vary in their potency and dosing regimens. While much research has been conducted investigating bioavailability and relative anti-inflammatory effects for these medications, most of these studies focused on ocular surface disease (Sendrowski et al. 2008). For effective treatment of anterior uveitis, the corticosteroid must penetrate the cornea and reach therapeutic levels within the anterior chamber. The size and chemical composition of the corticosteroid molecule, as well as the topical formulation (e.g., solution, suspension, or emulsion), are factors that may affect corneal penetration and access to the anterior chamber. Studies investigating topical dexamethasone administration have shown that suspensions penetrate the anterior chamber better than solutions (Cagini et al. 2016) and that vitreous concentrations of dexamethasone after hourly dosing were negligible (Weijtens et al. 2002).

Few well-conducted clinical trials exist comparing the efficacy of different topical corticosteroid formulations and doses in noninfectious anterior uveitis. Prednisolone acetate ophthalmic suspension 1.0% (Pred Forte, Allergan, Irvine, California) was shown to have superior anti-inflammatory activity in terms of resolving anterior chamber cell and flare compared to loteprednol etabonate ophthalmic suspension 0.5% (Lotemax, Pharmos Corporation and Bausch and Lomb Pharmaceuticals, Tampa, Florida) in patients with acute anterior uveitis (The Loteprednol Etabonate US Uveitis Study Group 1999). Fewer patients experienced IOP elevations $\geq$10 mmHg in the loteprednol etabonate group compared to the prednisolone acetate group; however, statistics comparing the groups were not provided. Difluprednate is a high-potency difluorinated prednisolone corticosteroid. The 0.5% difluprednate ophthalmic emulsion (Durezol, Alcon Laboratories, Fort Worth, TX) has high glucocorticoid receptor affinity, tissue penetration, and

bioavailability (Foster et al. 2010). Studies in patients with endogenous anterior uveitis have shown non-inferiority of difluprednate 0.05% dosed four times daily compared to prednisolone acetate 1% dosed eight times daily for 14 days (Foster et al. 2010; Sheppard et al. 2014). Clinically important IOP elevations were observed in 8.9–12% of eyes that received difluprednate compared to 3.7–5% of eyes that received prednisolone acetate 1%. However, other studies have reported IOP elevations $\geq$10 mmHg in 39–50% of eyes, with some eyes experiencing increases of over 30 mmHg (Birnbaum et al. 2011; Slabaugh et al. 2012). These studies also suggest that IOP elevation with topical difluprednate may be worse in the pediatric population.

As mentioned above, most topical corticosteroid formulations do not achieve sufficient concentrations in the posterior segment to be clinically useful for intermediate or posterior uveitis (Weijtens et al. 2002). The 0.5% difluprednate ophthalmic emulsion may be effective in treating some forms posterior uveitis (Onishi et al. 2015). In general, periocular or intravitreal injection of corticosteroids are required to achieve therapeutic levels in the posterior segment and are effective in the treatment of both active inflammation and macular edema (Sen et al. 2014). Clinically, these injections are most useful in cases of unilateral disease, in patients who are pseudophakic given the risk of cataract development, or in patients who are unable to tolerate systemic corticosteroids, such as poorly controlled diabetic patients.

Periocular treatments may be injected into the sub-Tenon's space or transcutaneously into the orbital floor. Typically, 40 mg of methylprednisolone or triamcinolone is injected (Ferrante et al. 2004). The half-life of triamcinolone after a single posterior sub-Tenon's injection in humans has been estimated at 25 days in the vitreous cavity (Shen et al. 2010). Injections can be repeated every 1–3 months as needed to control intraocular inflammation. In a retrospective review of 1,192 eyes that received at least one periocular corticosteroid injection, clinically meaningful cataract formation occurred in 20.2% of eyes (Sen et al. 2014). In the same study, intraocular pressure elevations to $\geq$24 and 30 mmHg occurred in 34% and 15% of eyes, respectively, and glaucoma surgery was required in 2.4% of eyes. Additional potential side effects of periocular corticosteroids injections include ptosis, orbital fat atrophy or prolapse, and inadvertent entrance into the globe (Lafranco Dafflon et al. 1999; Giles 1974; Dal Canto et al. 2005).

Intravitreal triamcinolone injection is another option for treating posterior segment inflammation or uveitic macular edema (Habot-Wilner et al. 2011). Typically, 2–4 mg of preservative-free triamcinolone is injected (Cunningham et al. 2008). Triamcinolone has been detected in the vitreous of non-vitrectomized eyes up to 2.75 months following a single 4 mg intravitreal injection (Mason et al. 2004). As with periorbital injections, intravitreal injections may be repeated for recurrent disease with close monitoring for side effects. In addition to development or progression of cataract and elevation of intraocular pressure, potential adverse effects of intravitreal steroid injections include vitreous hemorrhage, retinal detachment, as well as infectious or sterile endophthalmitis (Marticorena et al. 2012).

Two sustained release intravitreal corticosteroid implants have been approved by the US Food and Drug Administration (FDA) for the treatment of noninfectious uveitis. The dexamethasone intravitreal implant (Ozurdex, Allergan, Inc., Irvine, CA; 0.7 mg), which is administered in an office-based procedure, provides sustained release of dexamethasone via poly(lactic acid-co-glycolic acid) (PLGA) matrix material, which dissolves completely in vivo (Chang-Lin et al. 2011a). Studies in animal eyes have shown detection of dexamethasone in the vitreous and retina for up to 6 months, with peak concentrations in the first 2 months, without significant differences in concentrations between vitrectomized and non-vitrectomized eyes during the first month (Chang-Lin et al. 2011a, b). The 0.7 mg dexamethasone intravitreal implant was shown to significantly improve visual acuity and reduce vitreous haze scores in a multicenter randomized controlled clinical trial of patients with noninfectious intermediate or posterior uveitis compared to sham injection over 26 weeks (Lowder et al. 2011). 7.1% of eyes experienced intraocular pressure elevation $\geq$25 mmHg, and 15% of phakic eyes developed cataract. The median duration of therapeutic effect for first injections has been estimated to be 6 months, consistent with pharmacokinetic studies demonstrating detection of dexamethasone in the vitreous and retina for up to 6 months (Chang-Lin et al. 2011a; Tomkins-Netzer et al. 2014), and the mean time to second injections was estimated at 6.6 months (Zarranz-Ventura et al. 2014). Repeated insertions of the dexamethasone intravitreal implant have been reported without complication; (Querques et al. 2013) however, the number of applications that can be safely delivered to an eye remains unknown. Recently, results of a retrospective study showed the 0.7 mg dexamethasone intravitreal implant to be safe and effective in pediatric patients with noninfectious uveitis (Tomkins-Netzer et al. 2016).

The fluocinolone acetonide (FA) intravitreal implant (Retisert, 0.59 mg), which requires surgical implantation via sclerotomy in the operating room, provides sustained release of FA for approximately 30 months (Callanan et al. 2008). The Multicenter Uveitis Steroid Treatment (MUST) trial was designed to compare the efficacy of the FA intravitreal implant against systemic corticosteroid therapy, in addition to other systemic immunosuppressive medications when indicated, in patients with noninfectious intermediate uveitis, posterior uveitis, or panuveitis (Multicenter Uveitis Steroid Treatment Trial Research Group et al. 2010). Through 54 months of the study, visual acuity did not significantly differ between the groups at any time point; however, visual acuities were overall very good at the start of the trial, thereby limiting the potential for improvement with treatment (Multicenter Uveitis Steroid Treatment Trial Research group et al. 2015). While both treatments reduced the percentage of patients with active uveitis, the FA implant was significantly better at controlling inflammation at all time-points assessed. The FA implant was also significantly better at resolving macular edema through the first 2 years of treatment, after which systemic therapy showed equal efficacy. Cataract surgery was required significantly more often in the implant group through 54 months (87.7% vs 43% in the FA implant vs systemic treatment groups, respectively) with most surgeries occurring in the first 2 years (Multicenter Uveitis

Steroid Treatment Trial Follow-up Study Research Group 2015). Elevation of intraocular pressure and IOP-lowering surgeries (31.1% vs 4.5% in the FA implant vs systemic treatment groups, respectively, through 2 years) were also significantly more common in the implant. However, systemic adverse events were not different between the groups.

2.2 Systemic Corticosteroids

For severe cases of intraocular inflammation, especially bilateral disease, systemic treatment with corticosteroids is often employed. Oral prednisone at a dose of 1 mg/kg/day is a common starting dose, with gradual taper as inflammation subsides (Jabs et al. 2000). For particularly severe cases of sight-threatening ocular inflammation, such as Behçet's retinitis (Reed et al. 1998), corticosteroids may be administered intravenously (e.g., methylprednisolone dosed at 1,000 mg/day, which can be divided into four equal doses, for 3 days followed by high-dose oral prednisone with taper). Steroid-sparing immunosuppressive therapy (as discussed in following sections of this chapter) should be initiated if intraocular inflammation persists or recurs during steroid taper.

High doses of corticosteroids (e.g., 30 mg/day or more of prednisone) are associated with numerous adverse effects, and doses should be reduced as quickly yet safely as possible (Jabs et al. 2000). Adverse effects may occur anytime during corticosteroid treatment but are more common with higher doses and longer duration of use. Systemic side effects include, but are not limited to, osteoporosis, avascular necrosis, myopathy, hyperglycemia, weight gain, hypertension, hyperlipidemia, atherosclerosis, impaired wound healing, infection, psychological disturbance, and peptic ulcer disease. Supplementation with calcium and vitamin D should be prescribed to patients taking systemic corticosteroids, especially those on treatment for more than 3 months. Also, patients with a history of gastritis or gastroesophageal reflux disease or those who are concomitantly on non-steroidal anti-inflammatory drugs (NSAIDs) should be prescribed histamine-2 receptor blockers or proton pump inhibitors to reduce the risk of peptic ulcer disease.

3 T-Cell Inhibitors

Although corticosteroids remain a mainstay of treatment for uveitis, concern stemming from the side effects associated with their long-term use has prompted additional use of corticosteroid-sparing agents, such as the T-cell inhibitors cyclosporine and tacrolimus (Table 2). These agents decrease inflammation by interfering with signaling pathways involved in the function and proliferation of T cells (Knickelbein et al. 2015).

Table 2 Select steroid-sparing immunomodulatory medications used in the treatment of noninfectious uveitis

Class	Generic name	Mechanism	Type of molecule	Route	Typical dosing in uveitis
T-cell inhibitors	Cyclosporine A	Calcineurin inhibitor	Cyclic polypeptide isolated from the fungus *Beauveria nivea*	Oral	3–5 mg/kg/day divided into equal twice-daily doses
	Tacrolimus	Calcineurin inhibitor	Macrolide isolated from *Streptomyces tsukubaensis*	Oral	0.05–0.3 mg/kg/day
Antimetabolites	Methotrexate[a]	Dihydrofolate reductase inhibitor	Folic acid analog	Oral or subcutaneous	10–25 mg/week
	Azathioprine	Metabolized to 6-MP that blocks purine synthesis	Purine nucleoside analog	Oral	1–3 mg/kg/day divided into equal twice-daily doses
	Mycophenolate mofetil	Inosine monophosphate dehydrogenase inhibitor	Morpholinoethyl ester of mycophenolic acid	Oral	1,000–1,500 mg twice daily
Alkylating agents	Cyclophosphamide[a]	Cross-links DNA which blocks DNA replication	Nitrogen mustard derivative	Oral or intravenous (IV)	Oral: 1–3 mg/kg/day IV: 1 g/m^2 body surface area every 3-4 weeks
	Chlorambucil	Cross-links DNA which blocks DNA replication	Nitrogen mustard derivative	Oral	2 mg/day × 1 week, with an increase of 2 mg/day each week to 0.1–0.2 mg/kg/day
Biologic agents	Infliximab	Anti-TNFα	Chimeric (human/murine) monoclonal antibody	Intravenous	3–5 mg/kg at weeks 0, 2, and 6, then 3–10 mg/kg every 4–8 weeks
	Adalimumab	Anti-TNFα	Recombinant humanized monoclonal antibody	Subcutaneous	If <30 kg, 20 mg every 2–4 weeks; otherwise 40 mg every 1–2 weeks
	Golimumab	Anti-TNFα	Human monoclonal antibody	Subcutaneous	50 or 100 mg every 4 weeks
	Certolizumab	Anti-TNFα	Recombinant humanized monoclonal antibody fragment conjugated to polyethylene glycol	Subcutaneous	400 mg at weeks 0, 2, and 4 then 200 mg every 2 weeks

	Tocilizumab	Anti-IL6R	Recombinant humanized monoclonal antibody	Intravenous	4 mg/kg initial dose then 4–8 mg/kg every 4 weeks, not to exceed 800 mg per infusion
	Rituximab	Anti-CD20	Chimeric (human/murine) monoclonal antibody	Intravenous	500 or 1,000 mg at weeks 0 and 2, may repeat every 4–12 months

[a]Special considerations include addition of folic acid to the regimen for methotrexate and prophylactic use of Bactrim (to minimize the risk of infection with neutropenia), considering the use of Mesna (to avoid bladder toxicity) and counseling for fertility preservation for patients who plan on starting cyclophosphamide

3.1 Cyclosporine

Cyclosporine was first isolated as an antifungal agent in 1970 by Borel and co-workers in Switzerland (De Smet and Nussenblatt 1993). Although as an antifungal agent it proved to be too narrow in its spectrum of activity, it was incidentally found to have potential as an immunosuppressant through its effects on T cells. When cyclosporine enters T cells, it binds to cyclophilin A, increasing its affinity for calcineurin and thereby preventing calcineurin's ability to dephosphorylate proteins called nuclear factor of activated T cells (NFAT). Without dephosphorylation, NFAT proteins are unable to become activated and thus do not translocate to the nucleus, where they would normally influence the transcription of numerous inflammatory cytokines, including IL-2, IL-4, IL-10, IL-17, as well as tumor necrosis factor alpha (TNFα) and interferon gamma (IFN-γ) (Barbarino et al. 2013).

Initially, cyclosporine was primarily utilized to prevent and treat solid organ transplant rejections; however, in 1983, Nussenblatt et al. introduced a pilot study exhibiting the effectiveness of cyclosporine A in the treatment of ocular inflammation (Nussenblatt et al. 1983a), which was subsequently supported by several controlled and uncontrolled trials (Nussenblatt et al. 1983b, 1991; Masuda et al. 1989). More recently, a retrospective cohort study of 373 patients with noninfectious uveitis treated with cyclosporine in the Systemic Immunosuppressive Therapy for Eye Diseases Cohort Study (SITE Study) found that 51.9% of patients had achieved complete control of inflammation by 12 months and that corticosteroid-sparing success was achieved by 36.1% by 12 months. 8.2% achieved control without the need for systemic corticosteroids by 12 months (Kacmaz et al. 2010).

Although cyclosporine has been established as a useful alternative to corticosteroid monotherapy, its use has been limited due to concern for side effects associated with long-term use. Nephrotoxicity is one of the primary concerns, and even low doses of cyclosporine over the long term can significantly impair renal function, with decreases in glomerular filtration rate and irreversible kidney damage assessed by biopsy (Isnard Bagnis et al. 2002). Therefore, regular monitoring of serum creatinine and urea is essential, and patients should avoid concomitant NSAID use. Hypertension is seen in 15–20% of patients, and while it can be treated with antihypertensives, potassium-sparing diuretics should be avoided as cyclosporine may induce hyperkalemia (Kashani and Mearza 2008). Hepatotoxicity also may occur; however, it is of less concern as perturbations in liver enzymes are transient and patients tend to be asymptomatic. It also is important to monitor for the development of infection or malignancy (De Smet and Nussenblatt 1993), although Kempen and colleagues found that calcineurin inhibitors do not increase cancer risk to a degree that outweighs the expected benefits of therapy (Kempen et al. 2008).

Adult patients are treated with 3–5 mg/kg by mouth, divided into twice daily equal doses (Knickelbein et al. 2015). The dose should be decreased if blood pressure or creatinine levels rise, and treatment should be discontinued if values fail to normalize following dose adjustment. Children are also dosed at 3–5 mg/kg/day divided into two equal doses.

Cyclosporine use during pregnancy has been associated with increased rates of prematurity and other complications including preeclampsia (Bung and Molitor 1991); however, it does not appear to be a major teratogen (Bar Oz et al. 2001). Contraindications include severe infection, uncontrolled hypertension, or current malignancies (Kashani and Mearza 2008).

3.2 Tacrolimus

Tacrolimus was isolated from *Streptomyces tsukubaensis* in 1984 and is used widely to prevent rejection in solid organ transplant recipients (Gul et al. 2013). Like cyclosporine it functions as a T-cell inhibitor; however, it binds to the immunophilin FK-binding protein 12 rather than cyclophilin A. The downstream effects are the same, resulting in decreased transcription of inflammatory cytokines associated with T-cell activation (Barbarino et al. 2013). Although the mechanism of immunosuppression is similar, tacrolimus is thought to be associated with fewer adverse effects as lower doses are possible due to its increased potency up to 100 times that of cyclosporine (Barbarino et al. 2013).

In 1988, Kawashima and colleagues found that the capacity of tacrolimus to prevent experimental autoimmune uveitis induction in rats was 10–30 times more intense than that of cyclosporine (Kawashima et al. 1988). A decade later, another study examined the effects of low-dose tacrolimus in a small cohort of patients with endogenous posterior uveitis who had failed cyclosporine therapy and found that visual improvement was achieved for 3 months or more with a mean maintenance dose of 0.06 ± 0.02 mg/kg/day without development of nephrotoxicity, the primary reason for discontinuing cyclosporine A therapy (Kilmartin et al. 1998a). More recently, a randomized trial of tacrolimus versus cyclosporine in the treatment of posterior and intermediate uveitis also demonstrated similar efficacy with improved safety profile in tacrolimus versus cyclosporine (Murphy et al. 2005). Despite its improved safety profile, tacrolimus is associated with several adverse effects similar to cyclosporine, including nephrotoxicity, neurotoxicity, hypertension, gastrointestinal disturbances, infections, and malignancy (Barbarino et al. 2013). Both tacrolimus and cyclosporine also have been associated with the development of new-onset diabetes mellitus in renal transplant recipients; however, several studies have demonstrated improved glucose metabolism with cyclosporine compared to tacrolimus (Ghisdal et al. 2008; Mora 2010; Ramos-Cebrian et al. 2007; Wyzgal et al. 2003). This finding was further supported by a recent study of 67 patients with new-onset diabetes after renal transplantation randomized to receive either continuation of tacrolimus or conversion to cyclosporine (Rathi et al. 2015). HbA1c levels improved significantly only in the cyclosporine group, and the decline in fasting plasma glucose and insulin requirement was more significant in subjects on cyclosporine.

Tacrolimus therapy is typically started at a dose of 0.05–0.15 mg/kg/day with a maximum dose of 0.3 mg/kg/day (Jabs et al. 2000). While 95% of tacrolimus metabolites are removed via the biliary tract, renal excretion accounts for 2%

(Moller et al. 1999). Weekly laboratory assessments should include complete blood count and complete metabolic panel as well as assessment of blood pressure monthly at initiation of treatment and subsequently every 3 months after stable dosing has been achieved (Jabs et al. 2000).

Tacrolimus use during pregnancy has been associated with an increased risk of preterm birth and low birth weight; however, most studies involve its use in solid organ transplantations, and thus results may have been confounded by maternal condition (Nevers et al. 2014). According to the National Transplantation Pregnancy Registry, the incidence of major malformations associated with tacrolimus use was not much higher than in the general population (McKay and Josephson 2008). However, the available data are limited, and concerns have been raised about more subtle defects that may go unrecognized at birth, such as neurocognitive deficits. Calcineurin and FK-binding protein 12 are known to be increased in the fetal brain, and stimulation with tacrolimus may contribute to alterations in fetal cognitive development (Victor et al. 1995; Avramut et al. 2001).

4 Antimetabolites

Antimetabolites, including methotrexate, azathioprine, and mycophenolate mofetil, comprise another class of corticosteroid-sparing agents used in the treatment of ocular inflammation (Table 2). These drugs inhibit the proliferation of rapidly dividing cells, such as T and B lymphocytes, by antagonizing or competing with a metabolite needed for nucleotide synthesis (Kim and Foster 2006).

4.1 Methotrexate

Methotrexate was used initially as an antineoplastic agent in 1948 and for treatment of rheumatoid arthritis four decades later (Farber et al. 1948; Gangaputra et al. 2009). It was first implemented in the treatment of ocular inflammation in 1965 (Wong and Hersh 1965). Methotrexate functions as a potent inhibitor of dihydrofolate reductase, a key enzyme in the production of tetrahydrofolate, thereby decreasing the production of purines and pyrimidines required for DNA synthesis (Chan and Cronstein 2013). Just as it targets the rapidly proliferating cells of malignancy, its success in the treatment of uveitis is due to its ability to diminish the high turnover rate of inflammatory cells. In addition, methotrexate is thought to increase the rate of T-cell apoptosis and alter cytokine production (Wessels et al. 2008).

While methotrexate is most commonly administered orally, it can also be given via subcutaneous or intravitreal injections. When given orally, up to 35% of the dose is metabolized by intestinal flora prior to absorption; however, when parenterally administered, it is fully absorbed (Gangaputra et al. 2009; Larson et al. 2011). Gangaputra and colleagues retrospectively reviewed the records of 384 patients identified from the Systemic Immunosuppressive Therapy for Eye Diseases (SITE)

Study and found no significant difference in the effectiveness of subcutaneous versus oral routes of administration (Gangaputra et al. 2009). Among patients with anterior uveitis, intermediate uveitis, posterior or panuveitis, complete suppression of inflammation sustained for ≥28 days was reached in 55.6%, 47.4% and 38.6%, respectively, and corticosteroid-sparing success was achieved in 6 months among 46.1%, 41.3%, and 20.7%, respectively. When considering scleritis, ocular mucous membrane pemphigoid, and other forms of ocular inflammation in addition to uveitis, the overall success within 12 months was 66% and 58.4% for sustained control and corticosteroid-sparing, respectively.

Intravitreal administration of methotrexate was first used in the treatment of uveitis in 2006 and was found to achieve a faster onset of action than systemic administration (Hardwig et al. 2006; Taylor et al. 2009), which typically takes up to 6 months to reach its full effect (Gangaputra et al. 2009). When given intravitreally, the mechanism of action is thought to be primarily mediated by the release of adenosine into the extracellular space, ultimately inhibiting the activity of neutrophils, macrophages, and T lymphocytes (Cronstein et al. 1993; Chan and Cronstein 2002; Bouma et al. 1994; Constantin et al. 1998). The largest series to date of intravitreal methotrexate reported improvement in vision and control of inflammation in 79% of 38 eyes from 30 patients (Taylor et al. 2013). Furthermore, 73% of those who responded to treatment achieved a period of extended remission for a median of 17 months after a single intravitreal injection of methotrexate. However, adverse effects, including elevated intraocular pressure (Taylor et al. 2013) and corneal epitheliopathy (Smith et al. 2002), have been reported, and intravitreal methotrexate is rarely used in the routine management of noninfectious uveitis.

Systemic administration of methotrexate has the potential to cause several serious side effects, including hepatotoxicity, bone marrow suppression, and interstitial pneumonia (Jabs et al. 2000). Concomitant administration of folic acid can mitigate these effects at the recommended dose of 1 mg by mouth daily, excluding the day that methotrexate is taken (Knickelbein et al. 2015). Patients should also be advised to abstain from alcohol use during treatment. In addition to the side effects mentioned above, others more commonly seen include gastrointestinal upset with associated anorexia, nausea and vomiting, as well as stomatitis, alopecia, and rash (Jabs et al. 2000; Durrani et al. 2011).

Prior to initiation of therapy, the following should be obtained: complete blood count, serum chemistry profile, hepatitis B surface antigen, and hepatitis C antibody. Regular monitoring should occur every 1–2 months and should include complete blood count and liver function tests (Jabs et al. 2000). If liver enzymes are elevated to ≥2-times the upper limit of normal on two separate occasions, the dose should be reduced. Liver biopsy is warranted if enzyme abnormalities continue despite discontinuation of the drug. Treatment should be terminated if the following conditions occur: WBC <2,500 μl, platelet count <75,000/μl, or liver enzymes ≥5 times the upper limit of normal (Knickelbein et al. 2015).

In adults, systemic methotrexate is typically started at 2.5–10 mg/week, which is then increased to an average of 15 mg/week (ranging from 7.5 to 25 mg) after

several weeks if well tolerated (Jabs et al. 2000; Knickelbein et al. 2015). Intravitreal injections are dosed at 400 μg in 0.1 mL (Taylor et al. 2013). Guidelines for the dosage in children are less clear, but a recent systematic review and meta-analysis indicated 15 mg/m^2 was the most common dose based on body surface area (Simonini et al. 2013).

Methotrexate is a known teratogenic and abortive agent, and thus pregnancy and lactation should be avoided at any dose. Both men and women should be advised to discontinue treatment at least 3 months before attempting to conceive (Knickelbein et al. 2015; Visser et al. 2009).

4.2 Azathioprine

Azathioprine was introduced in the 1960s as an antileukemic agent (Elion 1989) and was soon utilized in solid organ transplantation (Murray et al. 1963; Danovitch 1999) as well as rheumatologic diseases such as systemic lupus erythematosus (Abu-Shakra and Shoenfeld 2001) and psoriatic arthritis (Lee et al. 2001). Its use in ophthalmic disease was first in the treatment of corneal graft rejection (Polack 1967) and later for noninfectious ocular inflammatory conditions (Pasadhika et al. 2009) such as active chronic iridocyclitis (Mathews et al. 1969), Behçet's disease (Yazici et al. 1990), and retinal vasculitis (Greenwood et al. 1998).

Azathioprine is a purine nucleoside analog that is metabolized to 6-mercaptopurine (6-MP), which after further metabolism can inhibit the first step in de novo purine-ring biosynthesis and ultimately become incorporated into replicating DNA and RNA, rendering it nonfunctional and thereby inhibiting the division and proliferation of inflammatory cells (Maltzman and Koretzky 2003). This mechanism targets lymphocytes due to their lack of a salvage pathway. Azathioprine is also thought to induce T-cell anergy or apoptosis through blockade of CD28 costimulation (Maltzman and Koretzky 2003; Elion 1993; Tiede et al. 2003).

Randomized clinical trials of azathioprine in ocular inflammation are limited and have largely focused on its use in Behçet's disease (Yazici et al. 1990; Hamuryudan et al. 1997). The SITE Study retrospectively reviewed the records of 145 patients, 63% of whom had uveitis, and found that 62% of patients initially gained complete control of inflammation sustained over at least 28 days within 1 year of therapy and 47% were able to maintain control while tapering systemic corticosteroids to $\leq$10 mg/day (Pasadhika et al. 2009). Patients with intermediate uveitis had the greatest rate of success, with 90% achieving sustained inflammatory inactivity within 1 year. However, when compared to other corticosteroid-sparing treatments for noninfectious ocular inflammation, azathioprine was found to have a longer median time to treatment success compared to mycophenolate mofetil (MMF) and a higher rate of side effects compared to both MMF and methotrexate (Galor et al. 2008).

Side effects associated with azathioprine most commonly include gastrointestinal intolerance, myelosuppression, and, less often, liver toxicity (Jabs et al. 2000;

Knickelbein et al. 2015; Clunie and Lennard 2004). Rarely, interstitial pneumonitis, pancreatitis, stomatitis, and alopecia have also been reported. Variations in metabolism of 6-MP can cause increased toxicity of the drug. For example, thiopurine methyltransferase (TPMT), a key enzyme in the methylation of 6-MP to an inactive metabolite, is controlled by a genetic polymorphism inherited as an autosomal codominant trait (Clunie and Lennard 2004; Weinshilboum and Sladek 1980). Decreased activity of TPMT leads to elevated cytotoxicity, possibly even within days of initiating azathioprine therapy (Clunie and Lennard 2004). Genetic testing or an assay of TPMT activity in red blood cells should be performed prior to starting treatment to allow for dose adjustment when necessary (Knickelbein et al. 2015; Durrani et al. 2011). Allopurinol, a strong xanthine oxidase (XO) and TPMT inhibitor, is known to interfere with the metabolism of azathioprine (Broekman et al. 2015). Consequently, the dose of azathioprine should be reduced by 25% for patients treated simultaneously with both drugs (Durrani et al. 2011).

Prior to initiating azathioprine, complete blood count with differential, serum creatinine, and liver enzymes should be obtained and repeated every 1–3 months throughout treatment (Jabs et al. 2000; Knickelbein et al. 2015; Durrani et al. 2011). In adults, 2 mg/kg/day is the most common dose, with ranges from 1 to 3 mg/kg/day. Patients are typically started at lower doses, and the dose is escalated if well tolerated. Doses can be given daily or twice daily when divided equally (Jabs et al. 2000; Knickelbein et al. 2015; Larson et al. 2011). Treatment should be discontinued if the following conditions occur: WBC $\leq$2,500/μl, platelet count $<$75,000/μl, liver enzymes $\geq$5 times the upper limit of normal, or an absolute neutrophil count below 1,000/μl (Knickelbein et al. 2015). If liver enzymes increase to $\geq$3 times the upper limit of normal, the dose should be reduced and liver enzymes should be retested 2 weeks later.

Azathioprine is pregnancy category D, meaning that the benefits of use during pregnancy may outweigh the potential teratogenic risks; however, most studies were based on its use in renal transplantation and inflammatory bowel disease (Caprilli et al. 2006; Ostensen and Förger 2013; Gerosa et al. 2014). A recent systematic review and meta-analysis on fetal outcomes after thiopurine use found that exposure in women was associated with preterm birth but not low birth weight or congenital abnormalities, and exposure in men at the time of conception was not associated with congenital abnormalities (Akbari et al. 2013). Nevertheless, due to the potential risk, contraception is important with the use of azathioprine and ideally, patients should not attempt to conceive for 3–4 months following the discontinuation of treatment (Knickelbein et al. 2015; Teruel et al. 2010).

4.3 Mycophenolate Mofetil

Mycophenolate mofetil (MMF) was initially introduced in 1946 as an antibiotic from *Penicillium brevicompactum* (Florey et al. 1946) and was first used as an immunosuppressant in the 1970s to treat psoriasis (Spatz et al. 1978). Two decades later it was utilized in solid organ transplant recipients as an alternative to other

immunosuppressive agents associated with undesirable side effects due to their non-selective antiproliferative mechanism (Allison and Eugui 1993). MMF is the prodrug of mycophenolic acid, an inhibitor of the rate-limiting enzyme in de novo synthesis of guanosine nucleotides, inosine monophosphate dehydrogenase (Allison and Eugui 2000). MMF preferentially inhibits the type II isoform of this enzyme, allowing it to specifically target activated lymphocytes, which express this form, thereby inhibiting the division and proliferation of inflammatory cells. By decreasing guanosine nucleotides, MMF also suppresses the expression of vascular endothelial adhesion molecules, which decreases recruitment of lymphocytes and monocytes to sites of inflammation.

MMF use in ocular inflammation was first explored in animal models of experimental autoimmune uveoretinitis (Chanaud et al. 1995), leading to a number of studies supporting its use in refractory human inflammatory eye diseases, including noninfectious uveitis and scleritis (Kilmartin et al. 1998b; Larkin and Lightman 1999; Sen et al. 2003; Greiner et al. 2002; Lau et al. 2003; Baltatzis et al. 2003; Siepman et al. 2006; Thorne et al. 2005; Teoh et al. 2008). Doycheva and colleagues conducted a retrospective case series of 60 uveitis patients treated with MMF for at least 5 years and found that control of inflammation was achieved in 72% of patients after 1 year of treatment and in 82% after 2 years (Doycheva et al. 2011). Rates of long-term side effects were similar to those reported in studies of short-term use (Siepman et al. 2006; Thorne et al. 2005). In a retrospective cohort study, MMF was found to have a more rapid time to control of ocular inflammation than methotrexate and an improved side effect profile compared to azathioprine (Galor et al. 2008). A recent randomized clinical trial in patients with noninfectious intermediate uveitis, posterior uveitis, or panuveitis did not find a statistically significant difference in corticosteroid-sparing control of intraocular inflammation between patients receiving mycophenolate mofetil or methotrexate; however, there was a trend toward higher treatment success in the methotrexate group (Rathinam et al. 2014). There was no difference in the time to treatment effect between mycophenolate mofetil and methotrexate.

Side effects associated with MMF most commonly include gastrointestinal upset, malaise, fatigue, headaches, and infection (Jabs et al. 2000; Doycheva et al. 2011). Bone marrow suppression and liver toxicities are less common but routine laboratory monitoring is essential and patients should limit alcohol consumption (Jabs et al. 2000; Knickelbein et al. 2015). In 2008, the FDA warned of the potential association of progressive multifocal leukoencephalopathy (PML) with MMF use (FDA 2008). However, further studies, including a retrospective cohort study of 32,757 renal transplant recipients as well as the SITE study with over 200 patients with ocular inflammation treated with MMF, failed to support this potential association (Daniel et al. 2010; Neff et al. 2008).

Prior to initiating therapy, complete blood count, serum creatinine, and liver function tests should be obtained and subsequently repeated every 1–3 months during treatment (Knickelbein et al. 2015; Durrani et al. 2011). In adults, orally administered MMF is typically initiated at 500 mg twice daily for 1–2 weeks, which is increased to 1 g twice daily if well tolerated. Once control of inflammation is

achieved, patients should continue therapy until they have been free of disease recurrences for 1–2 years. Treatment should be discontinued if the following conditions occur: WBC <2,500/μl, platelet count <75,000/μl, liver enzymes ≥5 times the upper limit of normal, or an absolute neutrophil count below 1,000/μl (Jabs et al. 2000). The dose should be reduced if liver function tests exceed ≥2–3-times the upper limit of normal or if there is a milder decrease in platelet count (Knickelbein et al. 2015). Patients should avoid simultaneous ingestion of antacids containing magnesium and aluminum hydroxide, as these reduce the bioavailability of MMF (Durrani et al. 2011).

MMF is a known teratogenic agent and has been found to decrease the effectiveness of oral contraceptives (Ostensen and Förger 2013; Gerosa et al. 2014; Sifontis et al. 2006). Therefore, two forms of contraception are needed, and male and female patients should avoid conception for at least the first 6 weeks but preferably 3–4 months after discontinuation of treatment (Knickelbein et al. 2015; Ostensen and Förger 2013).

5 Alkylating Agents

Alkylating agents, including cyclophosphamide and chlorambucil (Table 2), are derived from sulfur mustard or mustard gas, which was synthesized in 1860 and utilized in chemical warfare during the First World War (Frunzi 2007). In the 1940s, its ability to cause profound lymphopenia and myeloid suppression led to its introduction as a novel chemotherapeutic agent (Goodman et al. 1946). A decade later, the nitrogen mustard-derivative cyclophosphamide was first utilized in the treatment of ocular inflammation (Perez 1951), and another, chlorambucil, was added to the armamentarium in the 1970s (Patel et al. 2014). These agents inhibit the rapidly dividing cells of inflammation through disruption of DNA replication (Gallego-Pinazo et al. 2013). In general, their use has declined since the introduction of biologic agents.

5.1 Cyclophosphamide

Cyclophosphamide is comprised of a nitrogen mustard group attached to an oxazaphosphorine ring, which upon enzymatic activation functions as an alkylating agent to form DNA cross-links and DNA protein cross-links which inhibit DNA replication and lead to cell death (de Jonge et al. 2005). This results in a cytotoxic effect, particularly of the rapidly proliferating cells of malignancy as well as T and B lymphocytes involved in inflammation (Pujari et al. 2010). Thus, in addition to its use in chemotherapy, cyclophosphamide has also been utilized in the treatment of autoimmune diseases such as systemic lupus erythematosus and granulomatosis with polyarteritis (GPA, previously known as Wegener's granulomatosis) (Jabs et al. 2000). It was first introduced in the treatment of ocular inflammation in 1951 (Perez 1951).

Although cyclophosphamide has been found to be effective in the treatment of ocular inflammation, its risk of associated toxicities has limited its use. The SITE Study retrospectively reviewed the records of 215 patients with ocular inflammation and found that 49.2% and 76% of patients achieved sustained control of inflammation for at least 28 days within 6 and 12 months, respectively (Pujari et al. 2010). However, the authors cautioned that given the substantial risk of serious side effects, use of the drug should be limited to the most severe sight-threatening cases.

The most common side effects include reversible bone marrow suppression, nausea, vomiting, alopecia, and gonadal damage (de Jonge et al. 2005; Kruh and Foster 2012). Bladder injury potentially leading to hemorrhagic cystitis or malignant transformation is another concern and is thought to be due to the formation of acrolein, a highly reactive aldehyde metabolite excreted in the urine (Yazici et al. 1990; de Jonge et al. 2005; Cox 1979). To minimize the risk of bladder injury, patients should hydrate with 3–4 L of fluid per day to promote frequent voiding throughout the day (Knickelbein et al. 2015). In addition, patients should be advised to take cyclophosphamide in the morning to limit retention of harmful metabolites in the urine overnight (Monach et al. 2010). Sodium 2-mercaptoethane sulphonate (Mesna) may also be prescribed, as it binds to acrolein to promote its safe excretion (Manz et al. 1985). Increased risk of infection due to leukopenia can be treated prophylactically with trimethoprim-sulfamethoxazole (Bactrim) if needed (Jabs et al. 2000; Knickelbein et al. 2015). Other side effects include hepatic injury and interstitial pneumonitis (de Jonge et al. 2005). Concern has also been raised over possible increased risk of cutaneous malignancy as well as myeloproliferative disorders (Jabs et al. 2000; Yazici et al. 1990). Cyclophosphamide relies on the CYP enzymes for its degradation. Therefore, genetic polymorphisms of CYPs or concomitant use of drugs that inhibit CYPs may result in increased bioavailability and toxicity of the drug (de Jonge et al. 2005).

Cyclophosphamide may be administered orally or intravenously, and several studies have investigated whether pulsed IV delivery could offer rapid control of inflammation while avoiding prolonged bladder exposure and neutropenia (Wakefield 2014). Results have been conflicting, but most studies have reported IV therapy to be less effective than oral (Jabs et al. 2000; Rosenbaum 1994; Ozyazgan et al. 1992), while a small number have concluded that IV pulse alone or in combination with low-dose corticosteroid treatment is as effective as oral with fewer side effects and decreased mortality (Khan et al. 2013; Suelves et al. 2013). The SITE Study demonstrated a trend for increased cancer-related mortality, leading to the authors' suggestion that even though IV delivery may be less effective for inflammation control, it may be preferable in order to reduce the risk of malignancy (Kempen et al. 2008; Pujari et al. 2010; Martin et al. 1997).

The dosing of oral cyclophosphamide for ocular inflammation is typically 1–3 mg/kg/day (Jabs et al. 2000; Knickelbein et al. 2015; Larson et al. 2011; Yazici et al. 1990) and should be titrated for a target WBC of 3,000–4,000/mm^3 (Knickelbein et al. 2015). IV pulse therapy may be dosed at 1 g/m^2 body surface area every 3–4 weeks (Larson et al. 2011; Durrani et al. 2004). Upon initiation of

treatment, complete blood count, platelet count, and urine analysis should be checked weekly and eventually monthly, once values have stabilized (Knickelbein et al. 2015). Treatment should be discontinued if WBC falls below 2,500/mm^3 or if hematuria occurs, which should prompt a urology consult (Jabs et al. 2000; Knickelbein et al. 2015).

Cyclophosphamide is contraindicated in pregnancy as it is a known teratogen that has been associated with increased risk of skeletal and central nervous system abnormalities (Ostensen and Förger 2013). Lactation should also be avoided as the drug can be excreted in breast milk. Patients should be counseled on methods of fertility preservation as cyclophosphamide leads to infertility in both men and women due to disruption of oogenesis and spermatogenesis (Knickelbein et al. 2015). Simultaneous use of gonadotropin-releasing hormone treatment may increase the chance of continued fertility after completing treatment with cyclophosphamide (Knickelbein et al. 2015; Durrani et al. 2011; Blumenfeld and Haim 1997; Slater et al. 1999).

5.2 Chlorambucil

Chlorambucil is a nitrogen mustard alkylating agent introduced in 1953 as a more stable and less toxic derivative than cyclophosphamide (Miserocchi et al. 2002). Similar to cyclophosphamide, chlorambucil creates DNA cross-links that interfere with replication and transcription; however, its onset of action is slower (Larson et al. 2011). Although initially developed for treatment of malignancies, it was later utilized as an immunosuppressant to combat rheumatologic disorders. Since the 1970s, it has also been used to treat a variety of ocular inflammatory conditions such as Behçet's disease and sympathetic ophthalmia (Patel et al. 2014; Goldstein et al. 2002; Tessler and Jennings 1990).

The use of chlorambucil has been limited due to its potential to cause serious side effects such as bone marrow suppression, infections, sterility, and malignancy (Miserocchi et al. 2002). Other less common side effects include skin rash, gastrointestinal upset, nausea, vomiting, anorexia, and alopecia (Tessler and Jennings 1990; Godfrey et al. 1974; Andrasch et al. 1978). Unlike cyclophosphamide, it is not associated with hemorrhagic cystitis or malignant transformation of the bladder epithelium (Goldstein et al. 2002).

Several studies have demonstrated an increased risk of malignancy, particularly acute leukemia, associated with cumulative dose and duration of treatment (Khan et al. 1979; Berk et al. 1981; Palmer et al. 1984). Khan and colleagues retrospectively reviewed the records of 2006 patients treated for chronic inflammatory rheumatic conditions and found that development of acute leukemia was uncommon when duration of therapy was fewer than 6 months or total cumulative dose was less than 1.0 g (Khan et al. 1979). More recently, several studies have found that high-dose, short-term therapy may offer sustained control of inflammation while minimizing the risk of associated side effects (Patel et al. 2014; Goldstein et al. 2002). At the low doses used in long-term therapy, chlorambucil acts as an

inhibitor of protein synthesis, specifically of histones, while at high doses, it acts as a DNA alkylator leading to apoptosis (Sourlingas and Sekeri-Pataryas 1997). Disruption of histones causes structural instability and increased rate of mutations of the *p53* gene leading to secondary malignancies (Sturm et al. 2003). Thus, short-term high-dose therapy may offer a way to circumvent the process of malignant transformation. Although further studies are needed, the use of chlorambucil may be warranted in patients with severe disease refractory to other forms of treatment.

For ocular inflammation, chlorambucil is typically dosed at 0.1–0.2 mg/kg/day and continued for 1 year following control of inflammation (Knickelbein et al. 2015). Alternatively, high-dose therapy may be offered, consisting of 2 mg per day for 1 week followed by 2 mg per day each week until quiescence is achieved, the WBC count decreases to 2,400 cells per microliter or the platelet count drops below 100,000 cells per microliter (Larson et al. 2011; Tessler and Jennings 1990; Mamo 1976). Upon initiation of treatment, complete blood count should be checked weekly and eventually monthly, once values have stabilized.

Chlorambucil is a known teratogen and is contraindicated in pregnancy. Patients should be counseled on fertility preservation as it has been associated with testicular hypotrophy and azoospermia in men and premature ovarian failure in women (Patel et al. 2014; Blumenfeld et al. 2000).

6 Biologic Agents

Antibodies and other proteins that target specific components of the immune cascade to downregulate the immune response have become an important treatment modality for ocular inflammatory disease (Table 2), especially in cases of refractory uveitis or patient intolerance to conventional immunomodulatory therapy. In fact, an expert panel advocates using biologics as first-line agents in vision-threatening ocular Behçet's disease and second-line agents for many other types of chronic, vision-threatening ocular inflammatory disease (Levy-Clarke et al. 2014). Anti-tumor necrosis factor α (TNFα) agents are the most frequently used biologic agents for ocular inflammation, and other classes of biologic agents are emerging as potentially effective as well. The use of biologics for uveitis is considered off-label in the USA, given the lack of applicable randomized, controlled clinical trials. However, TNF inhibitors, for example, are approved for the treatment of uveitis in Japan and some European countries.

Since anti-inflammatory biologic agents downregulate the immune system, infections and an increased risk for malignancy are potential side effects. In addition to an increased frequency of upper respiratory infections, there is an increased risk for serious opportunistic infections and reactivation of latent infections. Current recommendations include screening for tuberculosis, hepatitis B, and hepatitis C prior to biologic administration (Selmi et al. 2015). Patients receiving biologic therapy should be vaccinated against influenza (inactivated vaccine), pneumococcal disease, and hepatitis B; these patients should not receive live vaccines. The biologic agents used in ocular inflammatory disease

have been developed relatively recently, so the long-term safety of these medications is unclear, especially given the possibility of an increased risk for malignancy.

Side effects that may limit biologic agent tolerance include systemic infusion reaction, injection site reaction, sustained liver function test abnormality, severe neutropenia, and severe thrombocytopenia. Also, cost is a major limitation to the use of biologic agents. Analysis of a US claims database using data from 2007 to 2011 showed that the average yearly cost per patient treated with an anti-TNFα agent ranged from $17,767 to $24,273, depending on the agent used (Schabert et al. 2013). Insurance companies differ in their coverage of these medications, especially for off-label use as in uveitis. Also, many biologic agents are administered intravenously, which further increases costs and inconveniences the patient by requiring visits to an infusion center. Notable exceptions that may be administered subcutaneously by the patient or a family member in a more flexible setting are adalimumab, golimumab, and certolizumab.

6.1 TNFα Antagonists

TNFα is a potent proinflammatory cytokine implicated in the primary pathogenesis of uveitis. TNFα levels are increased in both serum and aqueous of patients with active uveitis (Santos Lacomba et al. 2001). Intravitreal injection of TNFα in rabbits was shown to cause ocular inflammation by disrupting the blood-ocular barrier (Rosenbaum et al. 1988). Systemic TNFα administration conferred susceptibility to ocular inflammation in an experimental autoimmune uveitis (EAU) mouse model (Nakamura et al. 1994), and blocking TNFα in an EAU model suppressed the ocular inflammation (Sartani et al. 1996).

Anti-TNFα agents are biologically derived products first approved for the treatment of rheumatoid arthritis (Woodrick and Ruderman 2011) and now are the most commonly used biologic agents for uveitis (Levy-Clarke et al. 2014). Among the TNFα antagonists, infliximab has the most evidence for efficacy in ocular inflammatory disease, followed by adalimumab. Golimumab and certolizumab are newer anti-TNFα agents that also are potentially useful for treating ocular inflammatory disease. However, etanercept, the first anti-TNFα agent developed, is not recommended in uveitis. Substituting agents within the anti-TNFα class even if there is lack of response to the initial anti-TNFα agent may be beneficial in uveitis: a meta-analysis of anti-TNFα agent use in pediatric chronic autoimmune uveitis showed that among children who did not maintain disease remission with the initially prescribed anti-TNFα agent, 75% responded to a second anti-TNFα agent (Simonini et al. 2014).

Known multiple sclerosis (MS) is a contraindication to anti-TNFα therapy. Both patients in a case series of two patients with rapidly progressive MS treated with anti-TNFα antibody infusions showed increased numbers of gadolinium-enhancing brain lesions (van Oosten et al. 1996), and a randomized controlled clinical trial of

relapsing-remitting MS patients showed more frequent disease exacerbations in patients undergoing TNF blockade (Group TLMSSGaTUoBCMMA 1999).

Anti-TNFα therapy has been associated with other paradoxical autoimmune manifestations, including new cases of sarcoidosis with infliximab, adalimumab, etanercept, and certolizumab (Tong et al. 2012; Moisseiev and Shulman 2014). Psoriasiform rashes (Nguyen et al. 2013) and alopecia areata (Tauber et al. 2014) also have been reported with anti-TNFα therapy.

An early observational study suggested that the risk of lymphoma is higher in rheumatoid arthritis patients treated with anti-TNFα agents compared to conventional immunomodulatory therapy, but after subsequent analysis with an increased number of patients and follow-up duration the authors concluded that anti-TNFα agent use was not associated with an increased risk of lymphoma (Wolfe and Michaud 2007). Increased rates of lymphoma development have been found in other observational studies of TNFα antagonists compared to placebo (Geborek et al. 2005; Wong et al. 2012), but this finding is not consistent, and in all studies the number of lymphomas is small. Such analysis is further complicated by the increased risk of lymphoma in severe rheumatoid arthritis (Ekstrom et al. 2003). Although a theoretical risk for increased risk of various malignancies with biologic therapy persists, and further follow-up is indicated to definitively address this possibility, the studies to date do not support an increased risk for systemic malignancy.

The use of TNFα antagonists during pregnancy is generally avoided, but a prospective observational study showed no significant difference in the rate of congenital abnormalities in pregnancies with anti-TNFα exposure in the first trimester compared to disease-matched control pregnancies without anti-TNFα exposure and other pregnancies in normal controls (Diav-Citrin et al. 2014). TNFα antagonist exposure during early pregnancy may be less concerning since placental transfer of IgG antibodies is minimal during the first trimester, but placental transfer of IgG antibodies does become more efficient as pregnancy progresses, with case reports showing that infliximab is present in neonatal serum if the mother has been treated during the third trimester (Djokanovic et al. 2011).

Infliximab (Remicade, Janssen Biotech, Titusville, NJ, USA) is a chimeric (human-murine), monoclonal anti-TNFα IgG1k antibody. Multiple, relatively large case series show that infliximab is efficacious and well tolerated in Behçet's disease-associated uveitis (Arida et al. 2011; Calvo-Rio et al. 2014a; Takeuchi et al. 2014; Vallet et al. 2015), leading to the recommendation that it be considered a first-line agent for ocular Behçet's disease (Levy-Clarke et al. 2014).

Case series, observational studies, and open-label prospective clinical trials show that infliximab is efficacious for uveitis refractory to conventional immunosuppressive therapy in the context of scleritis, JIA-associated anterior uveitis, HLA-B27-associated anterior uveitis, ocular sarcoidosis, birdshot retinochoroidopathy, Vogt–Koyanagi–Harada disease (VKH), and idiopathic uveitis (Simonini et al. 2014; Murphy et al. 2004; Suhler et al. 2009; Sen et al. 2009; Ragam et al. 2014; Kruh et al. 2014). An open-label study examining the efficacy of infliximab for noninfectious uveitis refractory to at least one standard

immunosuppressive medication showed a 77% response rate at 10 weeks and a 48% response rate at 50 weeks (Suhler et al. 2009).

Adalimumab (Humira; AbbVie, North Chicago, IL, USA) is a fully humanized monoclonal anti-TNFα IgG1 antibody. Multiple studies show that adalimumab is efficacious in Behçet's-associated uveitis (Arida et al. 2011; Calvo-Rio et al. 2014a; Vallet et al. 2015; Díaz-Llopis et al. 2012), leading to the recommendation that it be considered a first-line agent for ocular Behçet's disease (Levy-Clarke et al. 2014).

Case series, observational studies, and open-label prospective clinical trials show that adalimumab can be efficacious for uveitis refractory to conventional immunosuppressive therapy in the context of scleritis, JIA-associated anterior uveitis, HLA-B27-associated anterior uveitis, tubulointerstitial nephritis and uveitis syndrome (TINU), ocular sarcoidosis, birdshot retinochoroidopathy, VKH, and idiopathic uveitis (Simonini et al. 2014; Ragam et al. 2014; Díaz-Llopis et al. 2012; Restrepo and Molina 2010; Suhler et al. 2013). An open-label study examining the efficacy of adalimumab for noninfectious uveitis refractory to at least one standard immunosuppressive medication showed a 68% response rate at 10 weeks and a 39% response rate at 50 weeks (Suhler et al. 2013).

An open question in uveitis is whether infliximab and adalimumab are equivalent in terms of efficacy and safety. Comparisons of early studies suggested that infliximab may be more effective but have more serious side effects than adalimumab (Knickelbein et al. 2015), but these comparisons were limited by small sample size and often were indirect. For instance, the prospective, open-label study of infliximab in refractory uveitis showed a more favorable response rate and more toxicity compared to adalimumab (Suhler et al. 2013), but these studies were not performed concurrently and thus were not designed to be directly compared. A systemic review and meta-analysis did not find a significant difference in response to infliximab versus adalimumab in pediatric chronic noninfectious uveitis (Simonini et al. 2014). A recent patient series examining outcomes in Behçet's disease showed no difference in efficacy or safety between infliximab and adalimumab (Vallet et al. 2015).

Golimumab (Simponi; Janssen Biotech, Titusville, NJ, USA) is a fully humanized monoclonal anti-TNFα antibody that first was reported to be useful for uveitis treatment in a series of two cases published in 2011 (Cordero-Coma et al. 2011). Additional case reports and retrospective case series show that golimumab can effectively control noninfectious intraocular inflammation (Faez et al. 2014; Miserocchi et al. 2014; Cordero-Coma et al. 2014; Calvo-Rio et al. 2014b). A recently published 3-year safety update shows that the safety profile of golimumab is similar to that of other TNFα antagonists (Kay et al. 2015).

Certolizumab pegol (Cimzia; UCB, Smyrna, GA, USA) is a humanized monoclonal anti-TNFα antibody Fab' fragment that has been PEGylated to prolong its half-life. Of the available anti-TNFα agents, certolizumab has the least amount of published data to document its efficacy in uveitis, although the available data are promising. The first case reporting efficacy of certolizumab in uveitis was published in 2015 and showed a clinical response in HLA-B27 spondylarthropathy-associated anterior uveitis with certolizumab, after failing infliximab and adalimumab (Maiz

Alonso et al. 2015). A recent case series showed that certolizumab had a response rate of 71.4% in cases of autoimmune uveitis that previously failed other anti-TNFα therapy (Llorenc et al. 2015).

Etanercept (Enbrel; Amgen, Thousand Oaks, CA, USA) is a recombinant fusion protein consisting of two copies of the soluble portion of the human TNF receptor fused to the human IgG1 Fc domain, and it downregulates TNFα signaling by binding free TNFα and preventing it from binding to cell surface TNFα receptors. Etanercept effectively treats inflammatory arthritis, but it appears to be less effective than other anti-TNFα agents in treating uveitis and scleritis (Smith et al. 2001, 2005; Doycheva et al. 2014; Galor et al. 2006). Etanercept use has been associated with new-onset uveitis and scleritis in challenge–dechallenge–rechallenge cases (Reddy and Backhouse 2003; Gaujoux-Viala et al. 2012), and a review of adverse drug events databases showed a significantly higher risk of uveitis with etanercept than with infliximab or adalimumab (Lim et al. 2007). As a result, etanercept use is avoided in uveitis patients, even in those with quiescent uveitis who may potentially benefit from its effects on inflammatory joint disease. This idea is evident in the prescribing patterns for juvenile idiopathic arthritis in the UK: the initially prescribed biologic agent in JIA patients with a history of chronic anterior uveitis is much more likely to be adalimumab or infliximab rather than etanercept, even though only etanercept and the interleukin-6 (IL-6) antagonist tocilizumab are approved for use in JIA by the National Institute for Health and Care Excellence (Kearsley-Fleet et al. 2016).

6.2 Anti-IL6 Agents

Elevated levels of IL-6, a proinflammatory cytokine, also have been demonstrated in serum (Kramer et al. 2007), aqueous (Murray et al. 1990), and vitreous (Perez et al. 2004) of uveitis patients. Yoshimura and colleagues have shown that IL-6 expression is necessary for ocular inflammation in an EAU mouse model and that IL-6 blockade ameliorates ocular inflammatory disease in that same EAU model (Yoshimura et al. 2009).

Tocilizumab (Actemra; Genentech, South San Francisco, CA, USA), a humanized monoclonal antibody that targets the IL-6 receptor, is becoming more widely used in noninfectious uveitis refractory to anti-TNFα treatment (Lin 2015), with case reports describing therapeutic success of tocilizumab in refractory birdshot chorioretinopathy (Muselier et al. 2011; Papo et al. 2014), idiopathic granulomatous panuveitis, juvenile idiopathic arthritis-associated uveitis (Tappeiner et al. 2012), Behçet's disease (Deroux et al. 2015), Castleman's disease-associated anterior uveitis and retinal vasculitis (Oshitari et al. 2012), and atypical Cogan's syndrome-associated anterior uveitis (Shibuya et al. 2013). Tocilizumab also may be effective in treating uveitic macular edema. Case series show improvement in refractory uveitic macular edema with tocilizumab (Mesquida et al. 2014), even in the absence of obvious active uveitis (Muselier et al. 2011; Deuter et al. 2016).

Safety studies of tocilizumab have shown an increased risk of serious infections comparable to that of anti-TNFα agents (Nishimoto et al. 2009), relatively uncommon and usually mild transfusion reactions, neutropenia, thrombocytopenia, transaminase elevations, and serum lipid elevations. Initial clinical trials identified 18 cases of gastrointestinal perforation in rheumatoid arthritis patients treated with tocilizumab (Gout et al. 2011). Subsequent analysis showed that gastrointestinal perforation occurred in the setting of diverticulitis in the majority of these cases, and the risk of gastrointestinal perforation with tocilizumab was not significantly different than the risk with anti-TNFα agents and was significantly lower than the risk with corticosteroids. Postmarketing studies in rheumatoid arthritis and juvenile idiopathic arthritis patients confirm that tocilizumab is well tolerated and identify opportunistic infections and bone marrow suppression with varying degrees of neutropenia and/or thrombocytopenia as the most common adverse effects (Genovese et al. 2013; Koike et al. 2014; Yokota et al. 2015).

Other anti-IL6 agents include clazakizumab (Alder BioPharmaceuticals, Bothell, WA, USA), olokizumab (UCB, Brussels, Belgium, and R-Pharm, Moscow, Russia), sarilumab (Regeneron Pharmaceuticals, Tarrytown, NY, USA), siltuximab (Janssen Biotech, Titusville, NJ, USA), and sirukumab (Janssen Biotech, Titusville, NJ, USA). Ongoing clinical trials assess the effectiveness of tocilizumab in juvenile idiopathic arthritis-associated uveitis (phase I/II); tocilizumab in noninfectious intermediate, posterior, and panuveitis (phase I/II); and sarilumab in noninfectious intermediate, posterior, and panuveitis (phase II) (clinicaltrials.gov).

6.3 Anti-CD20 Agents

Rituximab (Rituxan; Genentech, South San Francisco, CA, USA) is a chimeric (human/murine) monoclonal antibody against CD20, a cell surface molecule found only on the surface of mature B lymphocytes. A single treatment series, given as two infusions separated by 2 weeks, depletes mature B cells for 4–6 months. Of note, plasma cells do not express CD20 and thus are not depleted by rituximab. The exact mechanism of action for rituximab in autoimmune disease is unclear but may include prevention of plasma cell formation, alteration of B-cell–T-cell interactions, and/or diversion of immune effector cells toward rituximab–B-cell complexes and away from disease-specific immune complexes within affected tissues (Taylor and Lindorfer 2007). In addition to the side effects shared by the other biologic agents discussed in this chapter, a rare but daunting potential side effect of rituximab treatment is progressive multifocal leukoencephalopathy (PML) in the absence of human immunodeficiency virus infection. This potentially fatal outcome of JC virus reactivation occurred at an estimated rate of 1 in 25,000 in a rheumatoid arthritis population (Clifford et al. 2011).

A prospective interventional trial of 12 patients (Suhler et al. 2014) and a retrospective case series of 15 patients (Cao et al. 2016) both showed that rituximab can effectively treat noninfectious scleritis refractory to other treatment modalities. A pilot study in Behçet's disease showed that rituximab was more efficacious than

cytotoxic therapy (Davatchi et al. 2010). A case series showed that rituximab may achieve long-term quiescence in severe JIA-associated uveitis refractory to anti-TNFα agents (Miserocchi et al. 2015). Additionally, there are case reports documenting efficacy of rituximab in recalcitrant VKH (Caso et al. 2015) and diffuse subretinal fibrosis uveitis syndrome (Cornish et al. 2015). Of note, with its more favorable side effect profile, rituximab has essentially replaced cyclophosphamide in the treatment of GPA (Lally and Spiera 2015).

6.4 Other Biologic Agents

Other biologic agents that show promise in the treatment of ocular inflammatory disease include the anti-IL1 agents anakinra, canakinumab, and gevokizumab as well as the anti-IL17 agent secukinumab. Both IL-1β (Wan et al. 2016) and IL-17 (Amadi-Obi et al. 2007) are thought to be involved in the primary pathogenesis of human uveitis and EAU, with IL-1β acting upstream of IL-17. Case studies have shown efficacy of the recombinant IL-1 receptor antagonist anakinra (Kineret; Sobi, Stockholm, Sweden), the human monoclonal anti-IL1β antibody canakinumab (Ilaris; Novartis, Basel, Switzerland), and the human monoclonal anti-IL1β antibody gevokizumab (XOMA, Berkeley, CA, USA) in Behçet's disease; the potentially lower risk of tuberculosis reactivation with these medications compared to anti-TNFα agents suggests an advantage to using the anti-IL1 agents in tuberculosis-endemic areas (Cantarini et al. 2015). Studies of the human anti-IL17A monoclonal antibody secukinumab (Cosentyx; Novartis, Basel, Switzerland) in uveitis document both treatment success and treatment failure, perhaps because of differences in bioavailability based on administrative route. A recent randomized controlled trial of secukinumab in noninfectious active intermediate, posterior, or panuveitis showed an acceptable response rate of 72.7% and low relapse rates with high-dose intravenous administration; however, subcutaneous administration produced a response rate of only 33.3% (Letko et al. 2015).

7 Conclusions

Multiple therapeutic options currently exist for the treatment of noninfectious uveitis. Both local as well as systemic medications with various mechanisms of action may be utilized to combat intraocular inflammation. A common paradigm for treating chronic or recurrent noninfectious uveitis involves the "step-ladder" approach (Foster et al. 2016). This approach involves rapidly achieving disease quiescence with either local or systemic corticosteroids along with early initiation of traditional steroid-sparing therapy, such as mycophenolate, methotrexate, or cyclosporine. If these medications fail to control the disease, biologic agents are then added to the regimen. In especially recalcitrant cases, intraocular surgery in the form of pars plana vitrectomy may be indicated. As advancements are made in the understanding of inflammatory diseases, such as noninfectious uveitis, new

therapeutic targets will be discovered and additional treatment options will become available.

Acknowledgment This research was supported by the Intramural Research Program of the NIH, NEI.

References

The Loteprednol Etabonate US Uveitis Study Group (1999) Controlled evaluation of loteprednol etabonate and prednisolone acetate in the treatment of acute anterior uveitis. Loteprednol Etabonate US Uveitis Study Group. Am J Ophthalmol 127:537–544

Abu-Shakra M, Shoenfeld Y (2001) Azathioprine therapy for patients with systemic lupus erythematosus. Lupus 10:152–153

Akbari M, Shah S, Velayos FS, Mahadevan U, Cheifetz AS (2013) Systematic review and meta-analysis on the effects of thiopurines on birth outcomes from female and male patients with inflammatory bowel disease. Inflamm Bowel Dis 19:15–22

Allison AC, Eugui EM (1993) The design and development of an immunosuppressive drug, mycophenolate mofetil. Springer Semin Immunopathol 14:353–380

Allison AC, Eugui EM (2000) Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 47:85–118

Amadi-Obi A, Yu C-R, Liu X et al (2007) TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. Nat Med 13:711–718

Andrasch RH, Pirofsky B, Burns RP (1978) Immunosuppressive therapy for severe chronic uveitis. Arch Ophthalmol 96:247–251

Arida A, Fragiadaki K, Giavri E, Sfikakis PP (2011) Anti-TNF agents for Behcet's disease: analysis of published data on 369 patients. Semin Arthritis Rheum 41:61–70

Avramut M, Zeevi A, Achim CL (2001) The immunosuppressant drug FK506 is a potent trophic agent for human fetal neurons. Brain Res Dev Brain Res 132:151–157

Baltatzis S, Tufail F, Yu EN, Vredeveld CM, Foster CS (2003) Mycophenolate mofetil as an immunomodulatory agent in the treatment of chronic ocular inflammatory disorders. Ophthalmologe 110:1061–1065

Bar Oz B, Hackman R, Einarson T, Koren G (2001) Pregnancy outcome after cyclosporine therapy during pregnancy: a meta-analysis. Transplantation 71:1051–1055

Barbarino JM, Staatz CE, Venkataramanan R, Klein TE, Altman RB (2013) PharmGKB summary: cyclosporine and tacrolimus pathways. Pharmacogenet Genomics 23:563–585

Barnes PJ (2011) Glucocorticosteroids: current and future directions. Br J Pharmacol 163:29–43

Becker B, Mills DW (1963) Elevated intraocular pressure following corticosteroid eye drops. JAMA 185:884–886

Berk PD, Goldberg JD, Silverstein MN et al (1981) Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. N Engl J Med 304:441–447

Birnbaum AD, Jiang Y, Tessler HH, Goldstein DA (2011) Elevation of intraocular pressure in patients with uveitis treated with topical difluprednate. Arch Ophthalmol 129:667–668

Blumenfeld Z, Haim N (1997) Prevention of gonadal damage during cytotoxic therapy. Ann Med 29:199–206

Blumenfeld Z, Shapiro D, Shteinberg M, Avivi I, Nahir M (2000) Preservation of fertility and ovarian function and minimizing gonadotoxicity in young women with systemic lupus erythematosus treated by chemotherapy. Lupus 9:401–405

Bouma MG, Stad RK, van den Wildenberg FA, Buurman WA (1994) Differential regulatory effects of adenosine on cytokine release by activated human monocytes. J Immunol 153:4159–4168

Broekman MTJ, Roelofs HMJ et al (2015) Allopurinol and 5-aminosalicylic acid influence thiopurine-induced hepatotoxicity in vitro. Cell Biol Toxicol 31:161–171
Bung P, Molitor D (1991) Pregnancy and postpartum after kidney transplantation and cyclosporine therapy -- review of the literature adding a new case. J Perinat Med 19:397–401
Cagini C, Cometa F, Torroni G, Pellegrino A, Pellegrino R, Cavallini GM (2016) Dexamethasone disodium phosphate penetration into the human aqueous humor after topical application. Curr Eye Res 41(7):897–899
Callanan DG, Jaffe GJ, Martin DF, Pearson PA, Comstock TL (2008) Treatment of posterior uveitis with a fluocinolone acetonide implant: three-year clinical trial results. Arch Ophthalmol 126:1191–1201
Calvo-Rio V, Blanco R, Beltran E et al (2014a) Anti-TNF-alpha therapy in patients with refractory uveitis due to Behcet's disease: a 1-year follow-up study of 124 patients. Rheumatology 53:2223–2231
Calvo-Rio V, de la Hera D, Blanco R et al (2014b) Golimumab in uveitis previously treated with other anti-TNF-alpha drugs: a retrospective study of three cases from a single centre and literature review. Clin Exp Rheumatol 32:864–868
Cantarini L, Lopalco G, Caso F et al (2015) Effectiveness and tuberculosis-related safety profile of interleukin-1 blocking agents in the management of Behcet's disease. Autoimmun Rev 14:1–9
Cao JH, Oray M, Cocho L, Stephen Foster C (2016) Rituximab in the treatment of refractory non-infectious scleritis. Am J Ophthalmol 164:22–28
Caprilli R, Gassull MA, Escher JC, European Crohn's and Colitis Organisation et al (2006) European evidence based consensus on the diagnosis and management of Crohn's disease: special situations. Gut 55:i36–i58
Caso F, Rigante D, Vitale A et al (2015) Long-lasting uveitis remission and hearing loss recovery after rituximab in Vogt-Koyanagi-Harada disease. Clin Rheumatol 34:1817–1820
Chan ES, Cronstein BN (2002) Molecular action of methotrexate in inflammatory diseases. Arthritis Res 4:266–273
Chan ES, Cronstein BN (2013) Mechanisms of action of methotrexate. Bull Hosp Joint Dis 71 (Suppl 1):S5–S8
Chanaud NP 3rd, Vistica BP, Eugui E, Nussenblatt RB, Allison AC, Gery I (1995) Inhibition of experimental autoimmune uveoretinitis by mycophenolate mofetil, an inhibitor of purine metabolism. Exp Eye Res 61:429–434
Chang-Lin JE, Attar M, Acheampong AA et al (2011a) Pharmacokinetics and pharmacodynamics of a sustained-release dexamethasone intravitreal implant. Invest Ophthalmol Vis Sci 52:80–86
Chang-Lin JE, Burke JA, Peng Q et al (2011b) Pharmacokinetics of a sustained-release dexamethasone intravitreal implant in vitrectomized and nonvitrectomized eyes. Invest Ophthalmol Vis Sci 52:4605–4609
Clifford DB, Ances B, Costello C et al (2011) Rituximab-associated progressive multifocal leukoencephalopathy in rheumatoid arthritis. Arch Neurol 68:1156–1164
Clunie GPR, Lennard L (2004) Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine. Rheumatology 43:13–18
Constantin A, Loubet-Lescoulie P, Lambert N et al (1998) Antiinflammatory and immunoregulatory action of methotrexate in the treatment of rheumatoid arthritis: evidence of increased interleukin-4 and interleukin-10 gene expression demonstrated in vitro by competitive reverse transcriptase-polymerase chain reaction. Arthritis Rheum 41:48–57
Cordero-Coma M, Salom D, Diaz-Llopis M, Lopez-Prats MJ, Calleja S (2011) Golimumab for uveitis. Ophthalmology 118:1892.e3-4
Cordero-Coma M, Calvo-Rio V, Adan A et al (2014) Golimumab as rescue therapy for refractory immune-mediated uveitis: a three-center experience. Mediators Inflamm 2014:717598
Cornish KS, Kuffova L, Forrester JV (2015) Treatment of diffuse subretinal fibrosis uveitis with rituximab. Br J Ophthalmol 99:153–154

Cox PJ (1979) Cyclophosphamide cystitis--identification of acrolein as the causative agent. Biochem Pharmacol 28:2045–2049
Cronstein BN, Naime D, Ostad E (1993) The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. J Clin Invest 92:2675–2682
Cunningham MA, Edelman JL, Kaushal S (2008) Intravitreal steroids for macular edema: the past, the present, and the future. Surv Ophthalmol 53:139–149
Dal Canto AJ, Downs-Kelly E, Perry JD (2005) Ptosis and orbital fat prolapse after posterior sub-Tenon's capsule triamcinolone injection. Ophthalmology 112:1092–1097
Daniel E, Thorne JE, Newcomb CW et al (2010) Mycophenolate mofetil for ocular inflammation. Am J Ophthalmol 149:423–32.e1-2
Danovitch GM (1999) Choice of immunosuppressive drugs and individualization of immunosuppressive therapy for kidney transplant patients. Transplant Proc 31:2S–6S
Davatchi F, Shams H, Rezaipoor M et al (2010) Rituximab in intractable ocular lesions of Behcet's disease; randomized single-blind control study (pilot study). Int J Rheum Dis 13:246–252
de Jonge ME, Huitema AD, Rodenhuis S, Beijnen JH (2005) Clinical pharmacokinetics of cyclophosphamide. Clin Pharmacokinet 44:1135–1164
De Smet MD, Nussenblatt RB (1993) Clinical use of cyclosporine in ocular disease. Int Ophthalmol Clin 33:31–45
Deroux A, Chiquet C, Bouillet L (2015) Tocilizumab in severe and refractory Behcet's disease: four cases and literature review. Semin Arthritis Rheum. doi:10.1016/j.semarthrit.2015.11.012
Deuter CM, Zierhut M, Igney-Oertel A et al (2016) Tocilizumab in uveitic macular edema refractory to previous immunomodulatory treatment. Ocul Immunol Inflamm 5:1–6 (Epub ahead of print)
Diav-Citrin O, Otcheretianski-Volodarsky A, Shechtman S, Ornoy A (2014) Pregnancy outcome following gestational exposure to TNF-alpha-inhibitors: a prospective, comparative, observational study. Reprod Toxicol 43:78–84
Díaz-Llopis M, Salom D, Garcia-de-Vicuña C et al (2012) Treatment of refractory uveitis with adalimumab: a prospective multicenter study of 131 patients. Ophthalmology 119:1575–1581
Djokanovic N, Klieger-Grossmann C, Pupco A, Koren G (2011) Safety of infliximab use during pregnancy. Reprod Toxicol 32:93–97
Doycheva D, Zierhut M, Blumenstock G, Stuebiger N, Deuter C (2011) Long-term results of therapy with mycophenolate mofetil in chronic non-infectious uveitis. Graefes Arch Clin Exp Ophthalmol 249:1235–1243
Doycheva D, Zierhut M, Blumenstock G et al (2014) Immunomodulatory therapy with tumour necrosis factor alpha inhibitors in children with antinuclear antibody-associated chronic anterior uveitis: long-term results. Br J Ophthalmol 98:523–528
Durrani K, Papaliodis GN, Foster CS (2004) Pulse IV cyclophosphamide in ocular inflammatory disease: efficacy and short-term safety. Ophthalmology 111:960–965
Durrani K, Zakka FR, Ahmed M, Memon M, Siddique SS, Foster CS (2011) Systemic therapy with conventional and novel immunomodulatory agents for ocular inflammatory disease. Surv Ophthalmol 56:474–510
Ekstrom K, Hjalgrim H, Brandt L et al (2003) Risk of malignant lymphomas in patients with rheumatoid arthritis and in their first-degree relatives. Arthritis Rheum 48:963–970
Elion GB (1989) The purine path to chemotherapy. Science 244:41–47
Elion GB (1993) The George Hitchings and Gertrude Elion lecture. The pharmacology of azathioprine. Ann N Y Acad Sci 685:400–407
Faez S, Lobo AM, Sobrin L, Papaliodis GN (2014) Treatment of seronegative spondyloarthropathy-associated uveitis with golimumab: retrospective case series. Clin Experiment Ophthalmol 42:392–395
Farber S, Diamond LK, Mercer RD et al (1948) Temporary remissions in acute leukemia in children produced by folic antagonist 4-aminopteroylglutamic acid (aminopterin). N Engl J Med 238:787–793

FDA (2008) Communication about an ongoing safety review of CellCept (mycophenolate mofetil) and Myfortic (mycophenolate acid). http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/DrugSafetyInformationforHeathcareProfessionals/ucm072438.htm

Felinski EA, Cox AE, Phillips BE, Antonetti DA (2008) Glucocorticoids induce transactivation of tight junction genes occludin and claudin-5 in retinal endothelial cells via a novel cis-element. Exp Eye Res 86:867–878

Ferrante P, Ramsey A, Bunce C, Lightman S (2004) Clinical trial to compare efficacy and side-effects of injection of posterior sub-Tenon triamcinolone versus orbital floor methylprednisolone in the management of posterior uveitis. Clin Experiment Ophthalmol 32:563–568

Florey HW, Jennings MA et al (1946) Mycophenolic acid; an antibiotic from Penicillium brevicompactum Dlerckx. Lancet 1:46–49

Polack FM (1967) Effect of azathioprine (Imuran) on corneal graft reaction. Am J Ophthalmol 64:233–244

Foster CS, Davanzo R, Flynn TE, McLeod K, Vogel R, Crockett RS (2010) Durezol (Difluprednate Ophthalmic Emulsion 0.05%) compared with Pred Forte 1% ophthalmic suspension in the treatment of endogenous anterior uveitis. J Ocul Pharmacol Ther 26:475–483

Foster CS, Kothari S, Anesi SD et al (2016) The Ocular Immunology and Uveitis Foundation preferred practice patterns of uveitis management. Surv Ophthalmol 61:1–17

Frunzi J (2007) From weapon to wonder drug. Hospitalist

Gallego-Pinazo R, Dolz-Marco R, Martinez-Castillo S, Arevalo JF, Diaz-Llopis M (2013) Update on the principles and novel local and systemic therapies for the treatment of non-infectious uveitis. Inflamm Allergy Drug Targets 12:38–45

Galor A, Perez VL, Hammel JP, Lowder CY (2006) Differential effectiveness of etanercept and infliximab in the treatment of ocular inflammation. Ophthalmology 113:2317–2323

Galor A, Jabs DA, Leder HA et al (2008) Comparison of antimetabolite drugs as corticosteroid-sparing therapy for noninfectious ocular inflammation. Ophthalmology 115:1826–1832

Gangaputra S, Newcomb CW, Liesegang TL et al (2009) Methotrexate for ocular inflammatory diseases. Ophthalmology 116:2188–2198.e1

Gaujoux-Viala C, Giampietro C, Gaujoux T et al (2012) Scleritis: a paradoxical effect of etanercept? Etanercept-associated inflammatory eye disease. J Rheumatol 39:233–239

Geborek P, Bladstrom A, Turesson C et al (2005) Tumour necrosis factor blockers do not increase overall tumour risk in patients with rheumatoid arthritis, but may be associated with an increased risk of lymphomas. Ann Rheum Dis 64:699–703

Genovese MC, Rubbert-Roth A, Smolen JS et al (2013) Longterm safety and efficacy of tocilizumab in patients with rheumatoid arthritis: a cumulative analysis of up to 4.6 years of exposure. J Rheumatol 40:768–780

Gerosa M, Meroni PL, Cimaz R (2014) Safety considerations when prescribing immunosuppression medication to pregnant women. Expert Opin Drug Saf 13:1591–1599

Ghisdal L, Bouchta NB, Broeders N et al (2008) Conversion from tacrolimus to cyclosporine A for new-onset diabetes after transplantation: a single-centre experience in renal transplanted patients and review of the literature. Transpl Int 21:146–151

Giles CL (1974) Bulbar perforation during periocular injection of corticosteroids. Am J Ophthalmol 77:438–441

Godfrey WA, Epstein WV, O'Connor GR et al (1974) The use of chlorambucil in intractable idiopathic uveitis. Am J Ophthalmol 78:415–428

Goldstein DA, Fontanilla FA, Kaul S, Sahin O, Tessler HH (2002) Long-term follow-up of patients treated with short-term high-dose chlorambucil for sight-threatening ocular inflammation. Ophthalmology 109:370–377

Goodman LS, Wintrobe MM et al (1946) Nitrogen mustard therapy; use of methyl-bis (beta-chloroethyl) amine hydrochloride and tris (beta-chloroethyl) amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. JAMA 132:126–132

Gordon DM, Mc LJ, Koteen H et al (1951) The use of ACTH and cortisone in ophthalmology. Am J Ophthalmol 34:1675–1686
Gout T, Ostor AJ, Nisar MK (2011) Lower gastrointestinal perforation in rheumatoid arthritis patients treated with conventional DMARDs or tocilizumab: a systematic literature review. Clin Rheumatol 30:1471–1474
Greenwood AJ, Stanford MR, Graham EM (1998) The role of azathioprine in the management of retinal vasculitis. Eye 12:783–788
Greiner K, Varikkara M, Santiago C, Forrester JV (2002) Efficiency of mycophenolate mofetil in the treatment of intermediate and posterior uveitis. Ophthalmologe 99:691–694
Group TLMSSGaTUoBCMMA (1999) TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. Neurology 53:457–465
Gul FC, Turgut B, Dagli F, Ilhan N, Ozgen M (2013) The comparison of the impact of ghrelin and tacrolimus on vitreous cytokine levels in an experimental uveitis model. Graefes Arch Clin Exp Ophthalmol 251:1235–1241
Habot-Wilner Z, Sallam A, Pacheco PA, Do HH, McCluskey P, Lightman S (2011) Intravitreal triamcinolone acetonide as adjunctive treatment with systemic therapy for uveitic macular edema. Eur J Ophthalmol 21(Suppl 6):S56–S61
Hamuryudan V, Ozyazgan Y, Hizli N et al (1997) Azathioprine in Behçet's syndrome: effects on long-term prognosis. Arthritis Rheum 40:769–774
Hardwig PW, Pulido JS, Erie JC, Baratz KH, Buettner H (2006) Intraocular methotrexate in ocular diseases other than primary central nervous system lymphoma. Am J Ophthalmol 142:883–885
Isnard Bagnis C, Tezenas du Montcel S, Beaufils H et al (2002) Long-term renal effects of low-dose cyclosporine in uveitis-treated patients: follow-up study. J Am Soc Nephrol 13:2962–2968
Jabs DA, Rosenbaum JT, Foster CS et al (2000) Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel. Am J Ophthalmol 130:492–513
Jabs DA, Nussenblatt RB, Rosenbaum JT, Standardization of Uveitis Nomenclature Working Group (2005) Standardization of uveitis nomenclature for reporting clinical data. Results of the first international workshop. Am J Ophthalmol 140:509–516
Kacmaz RO, Kempen JH, Newcomb C et al (2010) Cyclosporine for ocular inflammatory diseases. Ophthalmology 117:576–584
Kashani S, Mearza AA (2008) Uses and safety profile of ciclosporin in ophthalmology. Expert Opin Drug Saf 7:79–89
Kawashima H, Fujino Y, Mochizuki M (1988) Effects of a new immunosuppressive agent, FK506, on experimental autoimmune uveoretinitis in rats. Invest Ophthalmol Vis Sci 29:1265–1271
Kay J, Fleischmann R, Keystone E et al (2015) Golimumab 3-year safety update: an analysis of pooled data from the long-term extensions of randomised, double-blind, placebo-controlled trials conducted in patients with rheumatoid arthritis, psoriatic arthritis or ankylosing spondylitis. Ann Rheum Dis 74:538–546
Kearsley-Fleet L, Davies R, Baildam E et al (2016) Factors associated with choice of biologic among children with Juvenile Idiopathic Arthritis: results from two UK paediatric biologic registers. Rheumatology (Oxford) 55(9):1556–1565
Keil JM, Liu X, Antonetti DA (2013) Glucocorticoid induction of occludin expression and endothelial barrier requires transcription factor p54 NONO. Invest Ophthalmol Vis Sci 54:4007–4015
Kempen JH, Gangaputra S, Daniel E, Levy-Clarke GA, Nussenblatt RB, Rosenbaum JT, Suhler EB, Thorne JE, Foster CS, Jabs DA, Helzlsouer KJ (2008) Long-term risk of malignancy among patients treated with immunosuppresive agents for ocular inflammation: a critical assessment of the evidence. Am J Ophthalmol 146:802–812
Khan MF, Arlet J, Bloch-Michel H et al (1979) Acute leukemias after treatment using cytotoxic agents for rheumatologic purpose. Nouv Presse Med 8:1393–1397

Khan IJ, Barry RJ, Amissah-Arthur KN et al (2013) Ten-year experience of pulsed intravenous cyclophosphamide and methylprednisolone protocol (PICM protocol) in severe ocular inflammatory disease. Br J Ophthalmol 97:1118–1122

Kilmartin DJ, Forrester JV, Dick AD (1998a) Tacrolimus (FK506) in failed cyclosporine A therapy in endogenous posterior uveitis. Ocul Immunol Inflamm 6:101–109

Kilmartin DJ, Forrester JV, Dick AD (1998b) Rescue therapy with mycophenolate mofetil in refractory uveitis. Lancet 352:35–36

Kim EC, Foster CS (2006) Immunomodulatory therapy for the treatment of ocular inflammatory disease: evidence-based medicine recommendations for use. Int Ophthalmol Clin 46:141–164

Knickelbein J, Jaworski L, Hasan J, Kaushal P, Sen HN, Nussenblatt RB (2015) Therapeutic options for the treatment of non-infectious uveitis. Expert Rev Ophthalmol 10:359–373

Koike T, Harigai M, Inokuma S et al (2014) Effectiveness and safety of tocilizumab: postmarketing surveillance of 7901 patients with rheumatoid arthritis in Japan. J Rheumatol 41:15–23

Kramer M, Monselise Y, Bahar I, Cohen Y, Weinberger D, Goldenberg-Cohen N (2007) Serum cytokine levels in active uveitis and remission. Curr Eye Res 32:669–675

Kruh J, Foster CS (2012) Corticosteroid-sparing agents: conventional systemic immunosuppressants. Dev Ophthalmol 51:29–46

Kruh JN, Yang P, Suelves AM, Foster CS (2014) Infliximab for the treatment of refractory noninfectious uveitis: a study of 88 patients with long-term follow-up. Ophthalmology 121:358–364

Lafranco Dafflon M, Tran VT, Guex-Crosier Y, Herbort CP (1999) Posterior sub-Tenon's steroid injections for the treatment of posterior ocular inflammation: indications, efficacy and side effects. Graefes Arch Clin Exp Ophthalmol 237:289–295

Lally L, Spiera R (2015) Current therapies for ANCA-associated vasculitis. Annu Rev Med 66:227–240

Larkin G, Lightman S (1999) Mycophenolate mofetil. A useful immunosuppressive in inflammatory eye disease. Ophthalmology 106:370–374

Larson T, Nussenblatt RB, Sen HN (2011) Emerging drugs for uveitis. Expert Opin Emerg Drugs 16:309–322

Lau CH, Comer M, Lightman S (2003) Long-term efficacy of mycophenolate mofetil in the control of severe intraocular inflammation. Clin Experiment Ophthalmol 31:487–491

Lee JC, Gladman DD, Schentag CT, Cook RJ (2001) The longterm use of azathioprine in patients with psoriatic arthritis. J Clin Rheumatol 7:160–165

Letko E, Yeh S, Foster CS, Pleyer U, Brigell M, Grosskreutz CL (2015) Efficacy and safety of intravenous secukinumab in noninfectious uveitis requiring steroid-sparing immunosuppressive therapy. Ophthalmology 122:939–948

Levy-Clarke G, Jabs DA, Read RW, Rosenbaum JT, Vitale A, Van Gelder RN (2014) Expert panel recommendations for the use of anti-tumor necrosis factor biologic agents in patients with ocular inflammatory disorders. Ophthalmology 121:785–796.e3

Lim LL, Fraunfelder FW, Rosenbaum JT (2007) Do tumor necrosis factor inhibitors cause uveitis? A registry-based study. Arthritis Rheum 56:3248–3252

Lin P (2015) Targeting interleukin-6 for noninfectious uveitis. Clin Ophthalmol 9:1697–1702

Llorenc V, Mesquida M, Sainz de la Maza M et al (2015) Certolizumab Pegol, a new anti-TNF-alpha in the armamentarium against ocular inflammation. Ocul Immunol Inflamm 24:167–172

Lowder C, Belfort R Jr, Lightman S et al (2011) Dexamethasone intravitreal implant for noninfectious intermediate or posterior uveitis. Arch Ophthalmol 129:545–553

Maiz Alonso O, Blanco Esteban AC, Egues Dubuc CA, Martinez Zabalegui D (2015) Effectiveness of certolizumab pegol in chronic anterior uveitis associated to Crohn's disease and ankylosing spondylitis. Reumatol Clin 11:189–190

Maltzman JS, Koretzky GA (2003) Azathioprine: old drug, new actions. J Clin Invest 111:1122–1124

Mamo JG (1976) Treatment of Behcet disease with chlorambucil. A follow-up report. Arch Ophthalmol 94:580–583
Manz I, Dietrich I, Przybylski M et al (1985) Identification and quantification of metabolite conjugates of activated cyclophosphamide and ifosfamide with mesna in urine by ion-pair extraction and fast atom bombardment mass spectrometry. Biomed Mass Spectrom 12:545–553
Marticorena J, Romano V, Gomez-Ulla F (2012) Sterile endophthalmitis after intravitreal injections. Mediators Inflamm 2012:928123
Martin F, Lauwerys B, Lefebvre C, Devogelaer JP, Houssiau FA (1997) Side-effects of intravenous cyclophosphamide pulse therapy. Lupus 6:254–257
Mason JO 3rd, Somaiya MD, Singh RJ (2004) Intravitreal concentration and clearance of triamcinolone acetonide in nonvitrectomized human eyes. Retina 24:900–904
Masuda K, Nakajima A, Urayama A et al (1989) Double masked trial of cyclosporin versus colchicine and long-term open study of cyclosporin in Behcet's disease. Lancet 1:1093–1096
Mathews JD, Crawford BA, Bignell JL, Mackay IR (1969) Azathioprine in active chronic iridocyclitis. A double-blind controlled trial. Br J Ophthalmol 53:327–330
McKay DB, Josephson MA (2008) Pregnancy after kidney transplantation. Clin J Am Soc Nephrol 3(Suppl 2):S117–S125
Mesquida M, Molins B, Llorenc V, de la Maza MS, Adan A (2014) Long-term effects of tocilizumab therapy for refractory uveitis-related macular edema. Ophthalmology 121:2380–2386
Miserocchi E, Baltatzis S, Ekong A, Roque M, Foster CS (2002) Efficacy and safety of chlorambucil in intractable noninfectious uveitis: the Massachusetts Eye and Ear Infirmary experience. Ophthalmology 109:137–142
Miserocchi E, Modorati G, Pontikaki I, Meroni PL, Gerloni V (2014) Long-term treatment with golimumab for severe uveitis. Ocul Immunol Inflamm 22:90–95
Miserocchi E, Modorati G, Berchicci L, Pontikaki I, Meroni P, Gerloni V (2015) Long-term treatment with rituximab in severe juvenile idiopathic arthritis-associated uveitis. Br J Ophthalmol. doi:10.1136/bjophthalmol-2015-306790
Moisseiev E, Shulman S (2014) Certolizumab-induced uveitis: a case report and review of the literature. Case Rep Ophthalmol 5:54–59
Moller A, Iwasaki K, Kawamura A et al (1999) The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. Drug Metab Dispos 27:633–636
Monach PA, Arnold LM, Merkel PA (2010) Incidence and prevention of bladder toxicity from cyclophosphamide in the treatment of rheumatic diseases: a data-driven review. Arthritis Rheum 62:9–21
Mora PF (2010) New-onset diabetes after renal transplantation. J Invest Med 58:755–763
Multicenter Uveitis Steroid Treatment Trial Follow-up Study Research Group (2015) Quality of life and risks associated with systemic anti-inflammatory therapy versus fluocinolone acetonide intraocular implant for intermediate uveitis, posterior uveitis, or panuveitis: fifty-four-month results of the Multicenter Uveitis Steroid Treatment Trial and Follow-up Study. Ophthalmology 122:1976–1986
Multicenter Uveitis Steroid Treatment Trial Research Group, Kempen JH, Altaweel MM, Holbrook JT, Jabs DA, Sugar EA (2010) The multicenter uveitis steroid treatment trial: rationale, design, and baseline characteristics. Am J Ophthalmol 149:550–561.e10
Multicenter Uveitis Steroid Treatment Trial Research Group, Kempen JH, Altaweel MM et al (2015) Benefits of systemic anti-inflammatory therapy versus fluocinolone acetonide intraocular implant for intermediate uveitis, posterior uveitis, and panuveitis: fifty-four-month results of the Multicenter Uveitis Steroid Treatment (MUST) Trial and Follow-up Study. Ophthalmology 122:1967–1975
Murphy CC, Ayliffe WH, Booth A, Makanjuola D, Andrews PA, Jayne D (2004) Tumor necrosis factor alpha blockade with infliximab for refractory uveitis and scleritis. Ophthalmology 111:352–356

Murphy CC, Greiner K, Plskova J et al (2005) Cyclosporine vs tacrolimus therapy for posterior and intermediate uveitis. Arch Ophthalmol 123:634–641
Murray JE, Merrill JP, Harrison JH, Wilson RE, Dammin GJ (1963) Prolonged survival of human-kidney homografts by immunosuppressive drug therapy. N Engl J Med 268:1315–1323
Murray PI, Hoekzema R, van Haren MA, de Hon FD, Kijlstra A (1990) Aqueous humor interleukin-6 levels in uveitis. Invest Ophthalmol Vis Sci 31:917–920
Muselier A, Bielefeld P, Bidot S, Vinit J, Besancenot J-F, Bron A (2011) Efficacy of tocilizumab in two patients with anti-TNF-alpha refractory uveitis. Ocul Immunol Inflamm 19:382–383
Nakamura S, Yamakawa T, Sugita M et al (1994) The role of tumor necrosis factor-alpha in the induction of experimental autoimmune uveoretinitis in mice. Invest Ophthalmol Vis Sci 35:3884–3889
Neff RT, Hurst FP, Falta EM et al (2008) Progressive multifocal leukoencephalopathy and use of mycophenolate mofetil after kidney transplantation. Transplantation 86:1474–1478
Nevers W, Pupco A, Koren G, Bozzo P (2014) Safety of tacrolimus in pregnancy. Can Fam Physician 60:905–906
Nguyen K, Vleugels RA, Velez NF, Merola JF, Qureshi AA (2013) Psoriasiform reactions to anti-tumor necrosis factor alpha therapy. J Clin Rheumatol 19:377–381
Nishimoto N, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, Azuma J (2009) Long-term safety and efficacy of tocilizumab, an anti-IL-6 receptor monoclonal antibody, in monotherapy, in patients with rheumatoid arthritis (the STREAM study): evidence of safety and efficacy in a 5-year extension study. Ann Rheum Dis 68:1580–1584
Nussenblatt RB, Whitcup SM (2010) Uveitis: fundamentals and clinical practice, 4th edn. Mosby/Elsevier, Amsterdam/Edinburgh
Nussenblatt RB, Palestine AG, Rook AH, Scher I, Wacker W, Gery I (1983a) Treatment of intraocular inflammatory disease with Cyclosporine A. Lancet 2:235–238
Nussenblatt RB, Palestine AG, Chan CC (1983b) Cyclosporine A therapy in the treatment of intraocular inflammatory disease resistant to systemic corticosteroids and cytotoxic agents. Am J Ophthalmol 96:275–282
Nussenblatt RB, Palestine AG, Chan CC, Stevens G Jr, Mellow SD, Green SB (1991) Randomized, double-masked study of cyclosporine compared to prednisolone in the treatment of endogenous uveitis. Am J Ophthalmol 112:138–146
Onishi SM, Asahi MG, Chou C, Gallemore RP (2015) Topical difluprednate for the treatment of Harada's disease. Clin Ophthalmol 9:157–167
Oshitari T, Kajita F, Tobe A et al (2012) Refractory uveitis in patient with castleman disease successfully treated with tocilizumab. Case Rep Ophthalmol Med 2012:968180
Ostensen M, Förger F (2013) How safe are anti-rheumatic drugs during pregnancy? Curr Opin Pharmacol 13:470–475
Ozyazgan Y, Yurdakul S, Yazici H et al (1992) Low dose cyclosporin A versus pulsed cyclophosphamide in Behcet's syndrome: a single masked trial. Br J Ophthalmol 76:241–243
Palmer R, Doré CJ, Denman AM (1984) Chlorambucil-induced chromosome damage to human lymphocytes is dose-dependent and cumulative. Lancet 1:246–249
Papo M, Bielefeld P, Vallet H et al (2014) Tocilizumab in severe and refractory non-infectious uveitis. Clin Exp Rheumatol 32:S75–S79
Pasadhika S, Kempen JH, Newcomb CW et al (2009) Azathioprine for ocular inflammatory diseases. Am J Ophthalmol 148:500–509.e2
Patel SS, Dodds EM, Echandi LV et al (2014) Long-term, drug-free remission of sympathetic ophthalmia with high-dose, short-term chlorambucil therapy. Ophthalmology 121:596–602
Perez R (1951) Case of uveitis of unknown etiology treated with nitrogen mustards. Rev Clin Esp 41:265–267
Perez V, Papaliodis G, Chu D, Anzaar F, Christen W, Foster C (2004) Elevated levels of interleukin 6 in the vitreous fluid of patients with pars planitis and posterior uveitis: the Massachusetts eye & ear experience and review of previous studies. Ocul Immunol Inflamm 12:205–214

Pujari SS, Kempen H, Newcomb CW, Gangaputra S, Daniel E, Suhler EB, Thorne JE, Jabs DA, Levy-Clarke GA, Nussenblatt RB, Rosenbaum JT, Foster CS (2010) Cyclophosphamide for ocular inflammatory diseases. Ophthalmology 117:356–365

Querques L, Querques G, Lattanzio R et al (2013) Repeated intravitreal dexamethasone implant (Ozurdex(R)) for retinal vein occlusion. Ophthalmologica 229:21–25

Ragam A, Kolomeyer AM, Fang C, Xu Y, Chu DS (2014) Treatment of chronic, noninfectious, nonnecrotizing scleritis with tumor necrosis factor alpha inhibitors. Ocul Immunol Inflamm 22:469–477

Ramos-Cebrian M, Torregrosa JV, Gutierrez-Dalmau A, Oppenheimer F, Campistol JM (2007) Conversion from tacrolimus to cyclosporine could improve control of posttransplant diabetes mellitus after renal transplantation. Transplant Proc 39:2251–2253

Rathi M, Rajkumar V, Rao N et al (2015) Conversion from tacrolimus to cyclosporine in patients with new-onset diabetes after renal transplant: an open-label randomized prospective pilot study. Transplant Proc 47:1158–1161

Rathinam SR, Babu M, Thundikandy R et al (2014) A randomized clinical trial comparing methotrexate and mycophenolate mofetil for noninfectious uveitis. Ophthalmology 121:1863–1870

Reddy AR, Backhouse OC (2003) Does etanercept induce uveitis? Br J Ophthalmol 87:925

Reed JB, Morse LS, Schwab IR (1998) High-dose intravenous pulse methylprednisolone hemisuccinate in acute Behcet retinitis. Am J Ophthalmol 125:409–411

Restrepo JP, Molina MP (2010) Successful treatment of severe nodular scleritis with adalimumab. Clin Rheumatol 29:559–561

Rosenbaum JT (1994) Treatment of severe refractory uveitis with intravenous cyclophosphamide. J Rheumatol 21:123–125

Rosenbaum JT, Howes EL Jr, Rubin RM, Samples JR (1988) Ocular inflammatory effects of intravitreally-injected tumor necrosis factor. Am J Pathol 133:47–53

Santos Lacomba M, Marcos Martín C, Gallardo Galera JM et al (2001) Aqueous humor and serum tumor necrosis factor-α in clinical uveitis. Ophthalmic Res 33:251–255

Sartani G, Silver PB, Rizzo LV et al (1996) Anti-tumor necrosis factor alpha therapy suppresses the induction of experimental autoimmune uveoretinitis in mice by inhibiting antigen priming. Invest Ophthalmol Vis Sci 37:2211–2218

Schabert VF, Watson C, Joseph GJ, Iversen P, Burudpakdee C, Harrison DJ (2013) Costs of tumor necrosis factor blockers per treated patient using real-world drug data in a managed care population. J Manag Care Pharm 19:621–630

Selmi C, Ceribelli A, Naguwa SM, Cantarini L, Shoenfeld Y (2015) Safety issues and concerns of new immunomodulators in rheumatology. Expert Opin Drug Saf 14:389–399

Sen HN, Suhler EB, Al-Khatib SQ, Djalilian AR, Nussenblatt RB, Buggage RR (2003) Mycophenolate mofetil for the treatment of scleritis. Ophthalmology 110:1750–1755

Sen HN, Sangave A, Hammel K, Levy-Clarke G, Nussenblatt RB (2009) Infliximab for the treatment of active scleritis. Can J Ophthalmol 44:e9–e12

Sen HN, Vitale S, Gangaputra SS et al (2014) Periocular corticosteroid injections in uveitis: effects and complications. Ophthalmology 121:2275–2286

Sendrowski DP, Jaanus SD, Semes LP, Stern ME (2008) Anti-inflammatory drugs. In: Bartlett JD, Jaanus SD (eds) Clinical ocular pharmacology, 5th edn. Butterworh-Heinemann, St. Louis, pp 222–224

Shen L, You Y, Sun S, Chen Y, Qu J, Cheng L (2010) Intraocular and systemic pharmacokinetics of triamcinolone acetonide after a single 40-mg posterior subtenon application. Ophthalmology 117:2365–2371

Sheppard JD, Toyos MM, Kempen JH, Kaur P, Foster CS (2014) Difluprednate 0.05% versus prednisolone acetate 1% for endogenous anterior uveitis: a phase III, multicenter, randomized study. Invest Ophthalmol Vis Sci 55:2993–3002

Shibuya M, Fujio K, Morita K, Harada H, Kanda H, Yamamoto K (2013) Successful treatment with tocilizumab in a case of Cogan's syndrome complicated with aortitis. Mod Rheumatol 23:577–581

Siepman K, Huber M, Stubiger N, Deuter C, Zierhut M (2006) Mycophenolate mofetil is a highly effective and safe immunosuppressive agent for the treatment of uveitis. Graefes Arch Clin Exp Ophthalmol 244:788–794

Sifontis NM, Coscia LA, Constantinescu S, Lavelanet AF, Moritz MJ, Armenti VT (2006) Pregnancy outcomes in solid organ transplant recipients with exposure to mycophenolate mofetil or sirolimus. Transplantation 82:1698–1702

Simonini G, Paudyal P, Jones GT, Cimaz R, Macfarlane GJ (2013) Current evidence of methotrexate efficacy in childhood chronic uveitis: a systematic review and meta-analysis approach. Rheumatology 52:825–831

Simonini G, Katie D, Cimaz R, Macfarlane GJ, Jones GT (2014) Does switching anti-TNFalpha biologic agents represent an effective option in childhood chronic uveitis: the evidence from a systematic review and meta-analysis approach. Semin Arthritis Rheum 44:39–46

Slabaugh MA, Herlihy E, Ongchin S, van Gelder RN (2012) Efficacy and potential complications of difluprednate use for pediatric uveitis. Am J Ophthalmol 153:932–938

Slater CA, Liang MH, McCune JW, Christman GM, Laufer MR (1999) Preserving ovarian function in patients receiving cyclophosphamide. Lupus 8:3–10

Smith JR, Levinson RD, Holland GN et al (2001) Differential efficacy of tumor necrosis factor inhibition in the management of inflammatory eye disease and associated rheumatic disease. Arthritis Rheum 45:252–257

Smith JR, Rosenbaum JT, Wilson DJ et al (2002) Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. Ophthalmology 109:1709–1716

Smith JA, Thompson DJS, Whitcup SM et al (2005) A randomized, placebo-controlled, double-masked clinical trial of etanercept for the treatment of uveitis associated with juvenile idiopathic arthritis. Arthritis Care Res 53:18–23

Sourlingas TG, Sekeri-Pataryas KE (1997) S and G2 phase histone biosynthesis of HEp-2 cells after the influence of the bisalkylating agent, chlorambucil. Biochem Mol Biol Int 42:1103–1114

Spatz S, Rudnicka A, McDonald CJ (1978) Mycophenolic acid in psoriasis. Br J Dermatol 98:429–435

Sturm I, Bosanquet AG, Hermann S, Guner D, Dorken B, Daniel PT (2003) Mutation of p53 and consecutive selective drug resistance in B-CLL occurs as a consequence of prior DNA-damaging chemotherapy. Cell Death Differ 10:477–484

Suelves AM, Arcinue CA, Gonzalez-Martin JM, Kruh JN, Foster CS (2013) Analysis of a novel protocol of pulsed intravenous cyclophosphamide for recalcitrant or severe ocular inflammatory disease. Ophthalmology 120:1201–1209

Suhler EB, Smith JR, Giles TR et al (2009) Infliximab therapy for refractory uveitis: 2-year results of a prospective trial. Arch Ophthalmol 127:819–822

Suhler EB, Lowder CY, Goldstein DA et al (2013) Adalimumab therapy for refractory uveitis: results of a multicentre, open-label, prospective trial. Br J Ophthalmol 97:481–486

Suhler EB, Lim LL, Beardsley RM et al (2014) Rituximab therapy for refractory scleritis: results of a phase I/II dose-ranging, randomized, clinical trial. Ophthalmology 121:1885–1891

Takeuchi M, Kezuka T, Sugita S et al (2014) Evaluation of the long-term efficacy and safety of infliximab treatment for uveitis in Behcet's disease: a multicenter study. Ophthalmology 121:1877–1884

Tappeiner C, Heinz C, Ganser G, Heiligenhaus A (2012) Is tocilizumab an effective option for treatment of refractory uveitis associated with juvenile idiopathic arthritis? J Rheumatol 39:1294–1295

Tauber M, Buche S, Reygagne P et al (2014) Alopecia areata occurring during anti-TNF therapy: a national multicenter prospective study. J Am Acad Dermatol 70:1146–1149

Taylor RP, Lindorfer MA (2007) Drug insight: the mechanism of action of rituximab in autoimmune disease--the immune complex decoy hypothesis. Nat Clin Pract Rheumatol 3:86–95

Taylor SR, Habot-Wilner Z, Pacheco P, Lightman SL (2009) Intraocular methotrexate in the treatment of uveitis and uveitic cystoid macular edema. Ophthalmology 116:797–801
Taylor SR, Banker A, Schlaen A et al (2013) Intraocular methotrexate can induce extended remission in some patients in noninfectious uveitis. Retina 33:2149–2154
Teoh SC, Hogan AC, Dick AD, Lee RW (2008) Mycophenolate mofetil for the treatment of uveitis. Am J Ophthalmol 146:752–760, 760.e1–3
Teruel C, Lopez-San Roman A, Bermejo F et al (2010) Outcomes of pregnancies fathered by inflammatory bowel disease patients exposed to thiopurines. Am J Gastroenterol 105:2003–2008
Tessler HH, Jennings T (1990) High-dose short-term chlorambucil for intractable sympathetic ophthalmia and Behcet's disease. Br J Ophthalmol 74:353–357
Thorne JE, Jabs DA, Qazi FA, Nguyen QD, Kempen JH, Dunn JP (2005) Mycophenolate mofetil therapy for inflammatory eye disease. Ophthalmology 112:1472–1477
Tiede I, Fritz G, Strand S et al (2003) CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. J Clin Invest 111:1133–1145
Tomkins-Netzer O, Taylor SR, Bar A et al (2014) Treatment with repeat dexamethasone implants results in long-term disease control in eyes with noninfectious uveitis. Ophthalmology 121:1649–1654
Tomkins-Netzer O, Talat L, Seguin-Greenstein S, Bar A, Lightman S (2016) Outcome of treating pediatric uveitis with dexamethasone implants. Am J Ophthalmol 161:110–115.e2
Tong D, Manolios N, Howe G, Spencer D (2012) New onset sarcoid-like granulomatosis developing during anti-TNF therapy: an under-recognised complication. Intern Med J 42:89–94
Urban RC Jr, Cotlier E (1986) Corticosteroid-induced cataracts. Surv Ophthalmol 31:102–110
Vallet H, Riviere S, Sanna A et al (2015) Efficacy of anti-TNF alpha in severe and/or refractory Behcet's disease: multicenter study of 124 patients. J Autoimmun 62:67–74
van Oosten BW, Barkhof F, Truyen L et al (1996) Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. Neurology 47:1531–1534
Victor RG, Thomas GD, Marban E, O'Rourke B (1995) Presynaptic modulation of cortical synaptic activity by calcineurin. Proc Natl Acad Sci U S A 92:6269–6273
Visser K, Katchamart W, Loza E et al (2009) Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. Ann Rheum Dis 68:1086–1093
Wakefield D (2014) Does cyclophosphamide still have a role in the treatment of severe inflammatory disease. Ocul Immunol Inflamm 22:306–310
Wan C-K, He C, Sun L, Egwuagu CE, Leonard WJ (2016) Cutting edge: IL-1 receptor signaling is critical for the development of autoimmune uveitis. J Immunol 196:543–546
Weijtens O, Schoemaker RC, Romijn FP, Cohen AF, Lentjes EG, van Meurs JC (2002) Intraocular penetration and systemic absorption after topical application of dexamethasone disodium phosphate. Ophthalmology 109:1887–1891
Weinshilboum RM, Sladek SL (1980) Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 32:651–652
Wessels JA, Huizinga TW, Guchelaar HJ (2008) Recent insights in the pharmacological actions of methotrexate in the treatment of rheumatoid arthritis. Rheumatology 47:249–255
Wolfe F, Michaud K (2007) The effect of methotrexate and anti-tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 person-years of observation. Arthritis Rheum 56:1433–1439
Wong VG, Hersh EM (1965) Methotrexate in the therapy of cyclitis. Trans Am Acad Ophthalmol Otolaryngol 69:279–293
Wong AK, Kerkoutian S, Said J, Rashidi H, Pullarkat ST (2012) Risk of lymphoma in patients receiving antitumor necrosis factor therapy: a meta-analysis of published randomized controlled studies. Clin Rheumatol 31:631–636

Woodrick RS, Ruderman EM (2011) Safety of biologic therapy in rheumatoid arthritis. Nat Rev Rheumatol 7:639–652

Woods AC (1950) Clinical and experimental observation on the use of ACTH and cortisone in ocular inflammatory disease. Am J Ophthalmol 33:1325–1349

Wyzgal J, Oldakowska-Jedynak U, Paczek L et al (2003) Posttransplantation diabetus mellitus under calcineurin inhibitor. Transplant Proc 35:2216–2218

Yazici H, Pazarli H, Barnes CG et al (1990) A controlled trial of azathioprine in Behçet's syndrome. N Engl J Med 322:281–285

Yokota S, Itoh Y, Morio T et al (2015) Tocilizumab in systemic juvenile idiopathic arthritis in a real-world clinical setting: results from 1 year of postmarketing surveillance follow-up of 417 patients in Japan. Ann Rheum Dis. doi:10.1136/annrheumdis-2015-207818

Yoshimura T, Sonoda KH, Ohguro N et al (2009) Involvement of Th17 cells and the effect of anti-IL-6 therapy in autoimmune uveitis. Rheumatology 48:347–354

Zarranz-Ventura J, Carreno E, Johnston RL et al (2014) Multicenter study of intravitreal dexamethasone implant in noninfectious uveitis: indications, outcomes, and reinjection frequency. Am J Ophthalmol 158:1136–1145.e5

Anti-angiogenic Therapy for Retinal Disease

Yannis M. Paulus and Akrit Sodhi

Contents

Abstract

Recent breakthroughs in our understanding of the molecular pathophysiology of retinal vascular disease have allowed us to specifically target pathological angiogenesis while minimizing damage to the neurosensory retina. This is

Y.M. Paulus
Kellogg Eye Center, University of Michigan School of Medicine, 1000 Wall Street, Ann Arbor, MI 48105, USA

A. Sodhi (✉)
Wilmer Eye Institute, Johns Hopkins University School of Medicine, 400 N. Broadway St., Smith Building, 4039, Baltimore, MD 21287, USA
e-mail: asodhi1@jhmi.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_78

perhaps best exemplified by the development of therapies targeting the potent angiogenic growth factor and vascular permeability mediator, vascular endothelial growth factor (VEGF). Anti-VEGF therapies, initially introduced for the treatment of choroidal neovascularization in patients with age-related macular degeneration, have also had a dramatic impact on the management of retinal vascular disease and are currently an indispensable component for the treatment of macular edema in patients with diabetic eye disease and retinal vein occlusions. Emerging evidence supports expanding the use of therapies targeting VEGF for the treatment of retinal neovascularization in patients with diabetic retinopathy and retinopathy of prematurity. However, VEGF is among a growing list of angiogenic and vascular hyperpermeability factors that promote retinal vascular disease. Many of these mediators are expressed in response to stabilization of a single family of transcription factors, the hypoxia-inducible factors (HIFs), that regulate the expression of these angiogenic stimulators. Here we review the basic principles driving pathological angiogenesis and discuss the current state of retinal anti-angiogenic pharmacotherapy as well as future directions.

Keywords

Angiogenesis • Hypoxia-inducible factor • Ischemia • Macular edema • Neovascularization • Oxidative stress • Vascular endothelial growth factor • Vascular permeability

1 Introduction

The development of the vascular system (i.e., vasculogenesis) occurs early during development and is an important foundational stage in organogenesis. In adults, the growth of new blood vessels (i.e., physiologic angiogenesis) is limited to wound healing and ovarian and cervical cycling (DiPietro 2016). Conversely, pathological angiogenesis is an essential component in disease, most notably in cancer, rheumatologic conditions including psoriasis, and ischemic disease (e.g., myocardial and cerebral infarction). The impact of pathological angiogenesis in disease is particularly evident for diseases of the retina, where it can lead to dramatic and permanent loss of vision. Pathological angiogenesis occurs in numerous settings including diabetic retinopathy, retinal vein occlusions, sickle cell retinopathy, hyperviscosity syndromes, pathologic myopia, age-related macular degeneration, retinal artery occlusion, radiation retinopathy, ocular ischemic syndrome, retinopathy of prematurity, familial exudative vitreoretinopathy (FEVR), retinal vasculitis, uveitic and inflammatory conditions, tumors, retinal vascular tumors including hemangiomas, retinal degeneration, post-traumatic changes, macular dystrophies, angioid streaks, choroidal rupture, Eales disease, sarcoidosis, chronic retinal detachment, carotid-cavernous fistula, Coats' disease, retinal artery macroaneurysms, and incontinentia pigmenti. Due to its easy accessibility and visualization, the retina is also an ideal model to study both vasculogenesis and angiogenesis: the retina is thin and

transparent; retinal vascular development is stereotyped both spatially and temporally; and vascularization of the retina occurs post-natally in rodents, the most commonly used vertebrate animals to study ocular disease.

Retinal laser therapy was developed over 50 years ago with investigations by Kapany and colleagues in 1963 using the ruby laser (Kapany et al. 1963) and has had a profound impact on the treatment of numerous retinal neovascular disorders. Seminal early studies established laser as the first treatment for numerous eye conditions. The Diabetic Retinopathy Study (DRS) established panretinal laser photocoagulation as the first effective treatment for high risk proliferative diabetic retinopathy (The Diabetic Retinopathy Study Research Group 1981). And the Early Treatment Diabetic Retinopathy Study (ETDRS) established focal laser as the first effective treatment for clinically significant diabetic macular edema (Early Treatment Diabetic Retinopathy Study Research Group 1991). The branch retinal vein occlusion study (BVOS) later demonstrated the efficacy of focal laser for the treatment of macular edema associated with branch vein occlusions (The Branch Vein Occlusion Study Group 1984). However, recent scientific discoveries have led to the development of new pharmacotherapies which have demonstrated significantly improved visual acuity outcomes with decreased side-effect profile. Indeed, the recent introduction of targeted pharmacologic anti-angiogenic therapies has transformed the care of patients with ocular neovascular diseases. This chapter will briefly describe the normal development of the retinal vasculature, the pathologic molecular events that lead to retinal and choroidal neovascularization, and currently available and possible future targets for anti-angiogenic therapy.

2 Embryology and Anatomy of the Retina

The development of the human eye has been described and refined over the past 90 years (Mann 1928). The outer layer of the optic cup evolves as a monolayer into the retinal pigment epithelium (RPE). The neurosensory retina develops from the inner layer of the neuroectodermal cells of the optic cup that begin to migrate in the second month of fetal development (Tripathi and Tripathi 1997). Vascular channels from the internal carotid artery develop in the mesenchyme around the optic vesicle late in the fourth week of gestation and the ophthalmic artery develops by the 6th week of gestation.

The most superficial retinal vascular layer first begins around 14 weeks gestation age as spindle-shaped undifferentiated mesenchymal cells arise from the hyaloid artery at the optic disc and develop into radial vessel extensions from the optic nerve head. Lumina develop behind the advancing edge of the mesenchymal cells. The vessels spread peripherally to reach the ora serrata just before term in centrifugal waves following neuronal differentiation with endothelial cells differentiating first followed by zonula occludens and gap junctions (Penfold et al. 1990). Deeper vascular layers arise sequentially by sprouting from the initial plexus (Michaelson 1948). The hyaloid system and tunica vasculosa lentis atrophy in the third trimester. The adult retinal vascular pattern occurs by remodeling through pruning and

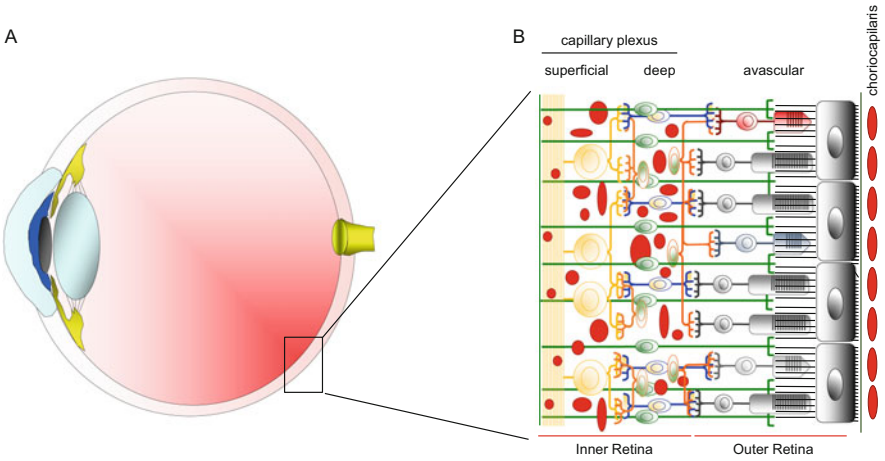

Fig. 1 Schematic of the eye and blood flow. (**a**) Schematic of the eye demonstrating the cornea, anterior chamber (*blue*), vitreous cavity (*red*), and retina (*black box*). (**b**) Retinal schematic diagram demonstrating juxtaposed retinal cells and the corresponding retinal and choroidal vasculature. The inner retina receives its blood supply from branches from the central retinal artery and has two distinct capillary plexi: the superficial capillary plexus within the ganglion cell layer and the deep capillary plexus within the inner nuclear layer. The outer retina, including the photoreceptors and retinal pigment epithelium, are supplied by the dense underlying vasculature of the choriocapillaris and choroid

maturation soon after formation by 3 months after birth. The foveal pit develops in the first 4 years of life.

The normal human circulatory system is made of several cell types. Endothelial cells make up the inner wall of the blood vessel. Inter-endothelial cell adhesions and tight junctions comprise important blood–tissue barriers. Smooth muscle cells in arterioles and pericytes in capillaries surround the endothelial cells and provide vascular tone and regulation. Glial cells such as astrocytes and retinal Müller cells are closely apposed to vessels in neural tissue. Dendritiform cells such as microglia are antigen presenting cells associated with vessels in certain tissues.

The human retina has 4 interconnected planar vascular networks in neuronal layers in the nerve fiber layer, ganglion cell layer, and inner nuclear layer. The retinal vessels form in alignment with the radial orientation of ganglion cell axons (Fig. 1). Importantly, the outer retina (the outer plexiform layer and photoreceptor layer) is avascular, and the macular pit (fovea) is also avascular at all times during human development (Fig. 2). The absence of vessels in the outer retina and fovea is essential for human vision.

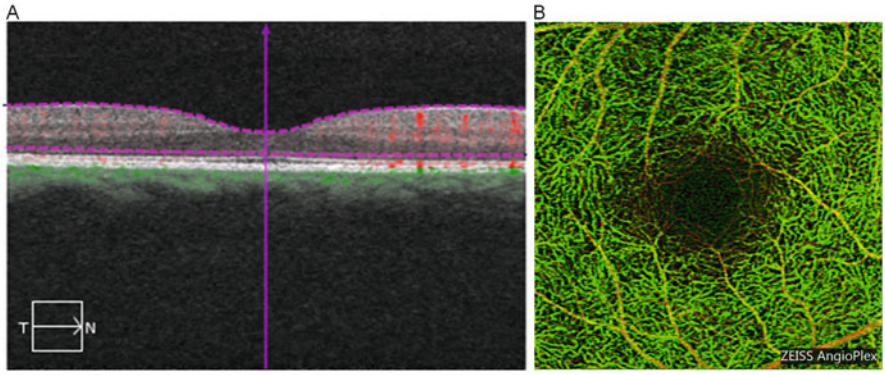

Fig. 2 Optical Coherence Tomography Angiography (OCTA). (**a**) OCT (Cirrus HD-OCT, Zeiss AngioPlex, Carl Zeiss Meditec, Inc., Dublin, CA) demonstrating calculations of the retinal thickness (*purple hashed lines*) and location of blood flow (*red*). (**b**) OCTA of a normal human retinal vasculature demonstrating the foveal avascular zone and surrounding vascular plexus. (Image courtesy of Gregory Hoffmeyer, Carl Zeiss Meditec, Dublin, CA)

3 Vasculogenesis

There are two primary modes of blood vessel assembly: vasculogenesis and angiogenesis. Vasculogenesis is the de novo assembly of vessels from endothelial precursor cells (angioblasts) and mesenchymal precursors that form clusters (blood islands). The cells aggregate into tubes that fuse, form tube-like structures, interconnect into cords, and slowly lumenize. Vasculogenesis forms the initial embryonic vessels and begins in gastrulation but plays a more limited role in adult vessel growth (McLeod et al. 1987; Hughes et al. 2000; Chan-Ling et al. 2004; McLeod et al. 2006).

In the developing human fetal retina, mesenchymal precursors precede the ingrowth of retinal blood vessels (Ashton 1970; Chan-Ling et al. 2004). Astrocytes are noted to be the majority of dividing cells in developing blood vessels in the retina (Sandercoe et al. 1999). Hemangioblasts serve as the precursor to angioblasts, which proliferate and migrate peripherally as a precursor to endothelial cells. Both astrocyte precursor cells and angioblasts have been described in developing fetal human retina (Chu et al. 2001; Chan-Ling et al. 2004). Once believed to happen exclusively in fetuses, new blood vessel formation due to angioblast differentiation has recently been demonstrated in pathological angiogenesis in adults (Jiang et al. 2005). In this setting, angioblasts stored in the bone marrow of adults are recruited to tissue to initiate vasculogenesis.

Vascular guidance and patterning during vessel growth occurs through attractant and repellant cues remarkably similar to axons in the developing nervous system. This requires a complex interplay between different cell types, including endothelial cells, pericytes, and periendothelial cells. Ganglion cells promote astrocyte

spread in a placental-derived growth factor (PDGF)-dependent manner. Astrocytes appear to serve as a template for vessels by secreting vascular endothelial growth factor (VEGF) and through the expression of cadherins to promote cellular adhesion. Vessels, in turn, downregulate expression of VEGF by astrocytes and induce astrocytic maturation. Early genetic and local tissue factors determine arterial versus venous fate of a plexus (Gariano 2003).

4 Angiogenesis

Angiogenesis is the growth of new vessels from pre-existing vessels by a sprouting or branching process. Angiogenesis elaborates on the vascular plexus begun with vasculogenesis and accounts for most vascular growth in adults, including pathologic vessel growth in disease. In stark contrast with normal retinal vasculogenesis, pathologic retinal angiogenesis creates chaotically oriented and physiologically deficient vessels that do not conform to neuronal histology. These vessels do not respect the normally avascular outer retina and macular pit, and can contribute to profound vision loss.

There are several steps in angiogenesis, including destabilization, vasodilation, hyperpermeability, cell migration, and cell proliferation. Each of these steps is regulated by the coordinated expression and/or activation of secreted mediators, cell-surface receptors, intracellular signaling molecules, and nuclear transcription factors. Circulating progenitor cells and hematopoietic stem cells from the bone marrow can contribute to angiogenesis, providing cellular building blocks for endothelial cells and secreting angiogenic molecules or differentiating into precursor perivascular cells. Angiogenesis is regulated by numerous physiologic properties including tissue oxygen tension, extracellular matrix composition, intrinsic genetic programs of endothelial cells, and a complex milieu of pro- and anti-angiogenic cells and molecules.

Vasodilation occurs in response to chemical signals (e.g., nitric oxide) as well as hyperpermeability factors (e.g., VEGF). Angiogenic growth factors (including VEGF) activate receptors on endothelial cells of pre-existing blood vessels. The vascular endothelial cells release proteases, including matrix metalloproteases (MMPs) that degrade the basement membrane, allowing vascular endothelial cells to escape from the parent vessel (Rodrigues et al. 2013), while plasminogen activator promotes the disruption of the endothelial cell junctions (Yao and Tsirka 2011). Migrating endothelial cells proliferate in the surrounding extracellular matrix towards the angiogenic stimulus using integrins and other adhesion molecules. Periendothelial cells and pericytes are then recruited to promote vessel maturity (Birbrair et al. 2014).

5 Additional Methods of New Vessel Development

Additional mechanisms of formation of blood vessels have also been described but play a more limited role in the retina. Intussusception involves the formation of transvascular tissue pillars and includes intussusceptive microvascular growth to expand a capillary network, intussusceptive arborization to form feeding vessels from capillaries, and intussusceptive branching remodeling through pillars arising close to a bifurcation or through pillars arising at some distance from the bifurcation point. Intussusception has been described in numerous organs including the eye, as well as lung, intestine, and kidney (Djonov et al. 2002; Burri et al. 2004). Tube formation and remodeling, vascular mimicry, and biochemical translocation and expansion have also been described. Another mechanism, hemo-vasculogenesis, has been described in which blood vessel and blood cells differentiate from a common precursor, the hemangioblast. This is thought to play a role in human choroidal vascular development from 6 until 22 weeks gestation and involves endothelial cells or angioblasts and erythroblasts in the choriocapillaris. Fenestrations, smooth muscle actin, extracellular basal lamina, and pericytes develop later (Lutty et al. 2010).

6 Hypoxia and the Retinal Vasculature

In 1948, Michaelson used dye-perfusion techniques to evaluate the embryonic and perinatal retinal vasculature. He found that capillaries grow extensively around venules and less around arteries. Michaelson posited a "Factor X," an oxygen-sensitive molecule, which controlled retinal vascular development through a concentration gradient (Michaelson 1948). Indeed, decreased oxygenation due to relative non-perfusion or an increase in metabolic demand is thought to drive the development of blood vessels in normal development (Yu and Cringle 2001).

The important role of oxygen in retinal vascular development has been further refined since the observations of Michaelson. The superficial vascular plexus in the developing retina follows the metabolic demand of neuronal development and physiologic hypoxia (Chan-Ling et al. 1995). Lowering the percentage of inhaled oxygen in animals reduces the rate and density of the retinal vasculature (Phelps 1990; Li et al. 2008). Hypoxia also plays an essential role in many retinal vascular diseases (Smith et al. 1994; Kaur et al. 2008), providing a link between physiologic and pathologic vascular processes in the eye. Indeed, the oxygen-dependent angiogenic stimulators expressed during normal vascular development are also expressed (albeit at much higher levels) in retinal vascular disease (Gariano and Gardner 2005).

7 Hypoxia-Inducible Factor (HIF)

A group of transcriptional activators, the hypoxia-inducible factors (HIFs), have recently emerged as the master regulators of these hypoxia-regulated angiogenic stimulators (Semenza 2012) and have helped illuminate the overlap between physiologic and pathologic vascular processes in the eye. HIFs are heterodimeric proteins composed of an exquisitely oxygen-sensitive α subunit and a ubiquitously expressed β subunit (Semenza 2007). HIF-1α was the first HIF α subunit isoform to be identified (Wang et al. 1995) and its role has been extensively studied in retinal vascular disease (Scholz and Taylor 2013). Two other isoforms, HIF-2α and HIF-3α have since been reported; while HIF-2α is closely related to HIF-1α and activates hypoxia-inducible gene transcription, HIF-3α is distantly related and may antagonize HIF-1α and HIF-2α (Ratcliffe 2007).

In normoxic conditions, HIF-1α is hydroxylated and ubiquited by the VHL E3 ubiquitin ligase, labeling it for degradation in the proteosome (Maxwell et al. 1999). In hypoxic conditions, the VHL hydroxylase is inhibited. Thus, in normal conditions, HIF-1α is produced but constantly degraded. Conversely, under conditions of hypoxia (e.g., in ischemic retinal disease) HIF-1α is no longer targeted for degradation, and can promote the expression of pro-angiogenic gene products, including VEGF. Indeed, VEGF expression is temporally and spatially correlated with HIF-1α expression and retinal vascular growth (Kurihara et al. 2014). HIF-1α regulates expression of the angiogenic template by astrocytes (Nakamura-Ishizu et al. 2012) and is essential for retinal vascular development (Caprara et al. 2011).

The precise molecular regulation of HIF-1α has been elegantly demonstrated. Under standard tissue culture conditions (20% O_2), proline residues 402 and 564 on the HIF-1α subunit are hydroxylated by a family of HIF prolyl hydroxylases (PHDs) (Kaelin 2005). Hydroxylated HIF-1α binds to the von Hippel-Lindau (VHL) tumor suppressor protein, which ubiquitinates HIF-1α and targets it for degradation by the proteasome (Hubbi et al. 2014; Mole et al. 2002). An additional level of regulation is provided by an asparaginyl hydroxylase, factor inhibiting HIF-1 (FIH-1) (Lando et al. 2002; Mahon et al. 2001). FIH-1 hydroxylates asparagine residue 803 on HIF-1α and prevents binding of the transcriptional co-activator, p300, to HIF-1α, thereby inhibiting its transcriptional activity.

Under hypoxic conditions (<5% O_2), the ability of PHDs and FIH to hydroxylate HIF-1α is impaired (Fig. 3). In the absence of hydroxylation, VHL does not bind to HIF-1α to trigger its degradation, whereas p300 binds to HIF-1α to enhance its transcriptional activity (Maxwell 2005; Schofield and Ratcliffe 2004; Manalo et al. 2005). The resulting increased amount of active HIF-1α protein localizes to the nucleus and binds to HIF-1β forming a heterodimer (HIF-1) that is capable of binding to the DNA of specific (hypoxia-inducible) genes, and inducing broad changes in gene expression that mediate acclimation of cells, tissues, and the organism to conditions of low oxygen tension (Semenza 2012). Indeed, HIF-1 targets include numerous genes that play essential adaptive roles by promoting angiogenesis to increase O_2 delivery, regulating the metabolic shift from oxidative

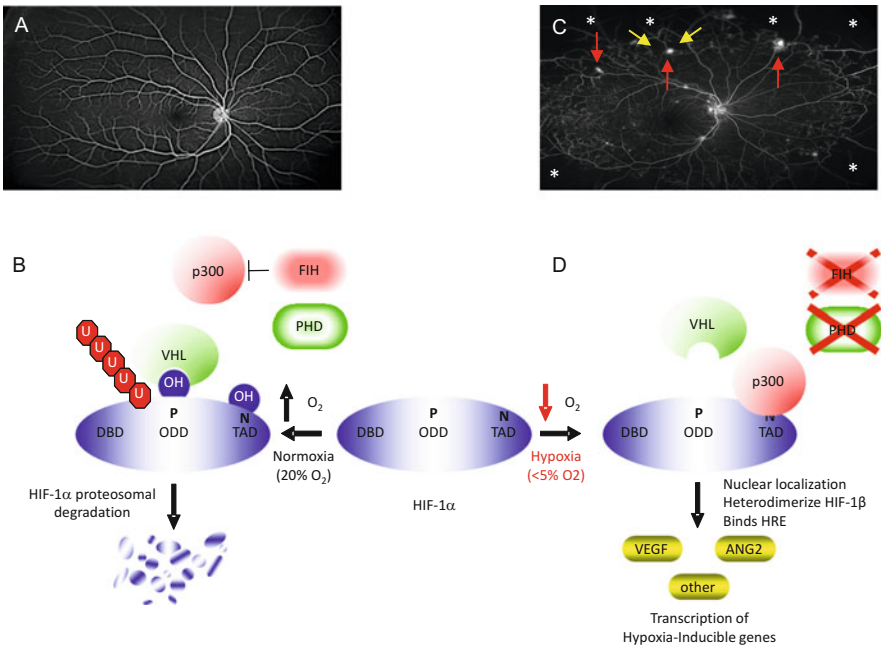

Fig. 3 Schematic of HIF-1α stabilization of the ischemic retina. (**a**) Ultrawide field fluorescein angiogram (Optos 200Tx, Optos plc, Dunfermline, Scotland, U.K.) of a normal retina. (**b**) Schematic of HIF-1α in normoxia demonstrating degradation involving von Hippel-Lindau (VHL) protein. HIF-1α is constitutively transcribed. In normoxia, HIF-1α is hydroxylated on proline and acetylated on lysine. This complex is preferentially bound by VHL, ubiquitinated, and degraded in the proteasome. (**c**) Ultrawide field fluorescein angiogram demonstrating extensive peripheral capillary non-perfusion (*star*) and microaneurysms along with regions of neovascularization (*red arrows*) adjacent to regions of capillary non-perfusion (*yellow arrows*). (**d**) Schematic of HIF-1α in hypoxia demonstrating HIF-induced transcription of hypoxia-inducible genes. In hypoxia, p300 is not inhibited by FIH (factor inhibiting HIF-1α) and interacts with the COOH-terminal transactivation domain (C-TAD). This results in HIF-1α stabilization against degradation through NH_2-terminal transactivation domain (N-TAD), resulting in nuclear localization, heterodimerization of HIF-1α and HIF-1β, and binding of hypoxia response elements (HRE). This results in the transcription of hypoxia-inducible genes, including vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang2)

phosphorylation to glycolysis and lactic acid production to decrease O_2 demand, protecting cells from acidosis, and influencing adaptive survival mechanisms (Semenza 2012). These genes work together to collectively promote the survival of cells exposed to hypoxia.

Numerous ischemic retinopathies exist where abnormal perfusion, permeability, and vasculature creates a mismatch between tissue metabolic demand and supply. These ischemic retinopathies involving retinal neovascularization include diabetic retinopathy, central and branch retinal vein occlusions, sickle cell retinopathy, hyperviscosity syndromes, radiation retinopathy, retinal tumors including retinoblastoma and uveal melanoma, ocular ischemic syndrome, chronic retinal

detachment, and retinopathy of prematurity. Proliferative diabetic retinopathy has been shown to result in an increase in numerous pro-angiogenic factors in the vitreous, including inflammatory cytokines, chemokines, extracellular matrix adhesion molecules, complement, polyamines, vasoactive peptides, and inflammatory cells (Gariano and Gardner 2005). Retinal oxygen saturation has been shown to decrease in patients with diabetes without retinopathy (Beach et al. 1999) and shown to increase adjacent to panretinal photocoagulation laser scars (Stefánsson et al. 1992). Retinal oxygen partial pressure has been shown to be reduced in cats with diabetes of 6–8 years duration (Linsenmeier et al. 1998). In addition to reduced oxygen, inflammation and aberrant wound-healing play an important role in diabetic retinopathy with microglial and glial cell activation resulting in the release of cytokines and chemokines (Amin et al. 1997; Krady et al. 2005).

In age-related macular degeneration (AMD), the development of choroidal neovascularization (CNV) appears to be a consequence of the expression of the same angiogenic mediators that drive the development of retinal neovascularization in ischemic retinal disease (Figg and Folkman 2008; Lin et al. 2012). However, it is postulated that oxidative stress instead of – or in addition to – hypoxia may stimulate accumulation of HIF-1α in AMD (Park et al. 2015). CNV occurs in conditions including age-related macular degeneration, pathologic myopia, uveitic and inflammatory conditions, choroiditis, presumed ocular histoplasmosis (POHS), punctate inner choroidopathy (PIC), multifocal choroiditis, angioid streaks, ocular trauma, vitelliform dystrophies, optic disc drusen, and idiopathic CNV. CNV occurs when the RPE or Bruch's membrane is compromised. Bruch's membrane is a five-layered extracellular matrix that separates the RPE from the choriocapillaris and is composed of the basement membrane of the RPE, inner collagenous zone, central band of elastic fibers, outer collagenous zone, and basement membrane of the choriocapillaris. AMD can develop CNV of the choroid in addition to retinal angiomatous proliferation (RAP) originating from the deep capillary bed of the retina.

8 Identifying Therapeutic Targets for the Treatment of Ocular Neovascular Disease

There are numerous considerations when identifying an ideal target for treatment of ocular neovascular disease. One would prefer a target whose expression is found only in the disease state (or markedly elevated in the diseased state). Low levels of the target in normal tissue would minimize potential side effects of inhibitory medications. In addition, one would prefer a secreted factor or transmembrane receptor that would be readily accessible to a binding molecule given the difficulty in achieving adequate intracellular or intranuclear concentrations of some medications. An ideal therapeutic agent would be administered locally to the eye in an easily accessible form, such as topical instillation, subconjunctival depot, or intravitreal. Administration would preferably be non-invasive, so that administration would involve minimal risk to patients. And the duration of action would

ideally be long, so as to minimize the number of re-treatments patients would need for these chronic diseases.

9 Vascular Endothelial Growth Factor (VEGF)

No single molecule has captured the attention of the retinal community as much as the angiogenic and vascular permeability stimulator, VEGF (Ferrara 2005). The VEGF family consists of VEGF-A, -B, -C, and -D, and placental-induced growth factor (PIGF) 1 and 2. VEGF is a hypoxia-inducible cytokine that is necessary for retinal vascularization and is spatially and temporally expressed in developing retinal blood vessels (Stone et al. 1995; Provis et al. 1997; Ozaki et al. 2000). VEGF plays numerous roles in the retina, including angiogenesis promotion, increasing vascular permeability, stimulating endothelial cell migration and proliferation, and increasing endothelial expression of plasminogen activator and interstitial collagenase. Mice expressing a single VEGF isoform demonstrate unique retinal vascular appearance, suggesting that each isoform acts within a limited spatial location to create the vasculature. Mice expressing VEGF164 appear normal, whereas mice expressing VEGF188 have aborted arterioles and normal veins (Stalmans et al 2002). VEGF120, a more diffusible version, results in reduced vessel branching. VEGF also plays an important role in vascular pruning in the developing retina. Relative hyperoxia surrounding arteries reduces VEGF production and its ability to maintain endothelial cell survival (Alon et al. 1995; Claxton and Fruttiger 2003). VEGF has been shown to be elevated in the eye in patients with proliferative diabetic retinopathy and retinal vein occlusions (Adamis et al. 1994; Aiello et al. 1994).

The VEGF receptors, VEGFR-1 or Flt-1, VEGFR-2 or Flk-1, VEGFR-3 or Flt-4, and neuropilin 1 and 2, are expressed in a temporally and spatially distinct pattern that also allows for vascular patterning (Shih et al. 2003; Favier et al. 2006; Gariano et al. 2006). Signals by a given receptor can elicit different responses at different sites. VEGFR-2 activation causes migration and filopodia in endothelial tip cells and proliferation in more proximal stalk cells (Gerhardt et al. 2003). Several inducing agents exist for VEGF, including TGF-α, TGF-β, PDGF, and hypoxia. VEGF also further refines vascular development through its interactions with other angiogenic factors, including placental growth factors, angiotensin, angiopoietin, and pigment epithelium-derived factor (Dawson et al. 1999; Feeney et al. 2003; Sarlos et al. 2003). Another potential use of anti-VEGF treatment is in the "normalization" of vasculature, reducing hypoxia and creating a window for chemotherapy (Datta et al. 2015).

Several clinical trials currently exist utilizing VEGF (Table 1). The first approved therapy for age-related macular degeneration (AMD) in December 2004 was pegaptanib sodium (Macugen, EyeTech Pharmaceuticals/Pfizer), an RNA aptamer directed against VEGF-165. Pegaptanib was the first aptamer approved for use in humans (Ng et al. 2006). Aptamers are oligonucleotide ligands with high-affinity binding to molecular targets. Pegaptanib was demonstrated to be safe and

Table 1 Current VEGF/KDR (kinase-insert-domain-containing receptor) anti-angiogenic targets in clinical trials

Drug	Drug target	Disease target	Clinicaltrials.gov ID	Phase	Number patients	Relevant clinical study
Pazopanib	VEGFR tyrosine kinase inhibitor	AMD	NCT00612456 NCT01134055	2, Completed 2009 2b	70 510	Danis et al. (2014)
TG100801	VEGF tyrosine kinase inhibitor	AMD, PDR	NCT00414999	1, Completed	44	
TG100801	Same	AMD	NCT00509548	2, Terminated early	Terminated after 7 due to corneal deposits	
OPT-302	VEGFR-3	AMD	NCT02543229	1	Enrolling 50	
Regorafenib	Receptor tyrosine kinase VEGFR2-TIE2	AMD	NCT02222207	2	52	
rAAV.sFlt-1	Flt1	AMD	NCT01494805	1 and 2	40	Rakoczy et al. (2015)
PAN-90806	Anti-VEGFR	AMD	NCT02022540	1	50	
Squalamine lactate	VEGF	AMD RVO PDR	NCT01678963 NCT02614937 NCT01769183	2 1 and 2 2	142 20 6	
Abicipar Pegol (AGN-150998)	Anti-VEGF	AMD DME	NCT01397409 NCT02181517 NCT02181504 NCT02462928 NCT02186119	2 2 2 3 2	271 25 25 900, R 151	
PF582	Anti-VEGF	AMD	NCT02121353	1 and 2	25	
EYE001	Anti-VEGF Pegylated Aptamer	AMD	NCT00021736	2 and 3	540	
AL-39324	Inhibit VEGFR and PDGFR	AMD	NCT00992563	2	35	

LHA510	VEGFA-inhibitor	AMD	NCT02076919 NCT02355028	1 2	135	
X-82	VEGF and PDGF inhibitor	AMD	NCT02348359 NCT01674569	2, R 1 and 2	132 35	
ESBA1008	Anti-VEGF	AMD	NCT01304693	1 and 2	376	
BCD-021	Anti-VEGF	AMD	NCT02450981	1	10	
LMG324	Anti-VEGF	AMD	NCT02398500	1 and 2	25, Terminated	
TK001	Anti-VEGF	AMD	NCT02613559	1	27,R	
ORA102		AMD	NCT00745511	1 and 2	96	
RG7716	Anti-VEGFA	AMD	NCT02484690	2	271	
AGN211745	VEGFR-1 siRNA	AMD	NCT00395057	2	138, Terminated	
PTK787 (Vatalanib)	Tyrosine kinase inhibitor targets VEGFR	AMD	NCT00138632	1 and 2	50	

Abbreviations: *R* recruiting, *AMD* neovascular age-related macular degeneration, *DME* diabetic macular edema, *PDR* proliferative diabetic retinopathy, *RVO* retinal vein occlusion

effective in several prospective, randomized, multicenter, double-masked, sham-controlled studies (Gragoudas et al. 2004; Chakravarthy et al. 2006; D'Amico et al. 2006).

Bevacizumab (Avastin, Genentech/Roche) was approved by the United States Food and Drug Administration (FDA) in 2004 as treatment for metastatic colon cancer and is a whole humanized from mouse antibody to VEGF-A (Cohen et al. 2007). It was described for use initially systemically as an intravenous treatment (Michels et al. 2005) and subsequently via off-label intravitreal administration for AMD (Avery et al. 2006; Rich et al. 2006). Prospective randomized controlled trials have proven the efficacy of bevacizumab in treating AMD (Sacu et al. 2009; Tufail et al. 2010), macular edema from CRVO (Epstein et al. 2012), and diabetic macular edema (DME) in the BOLT trial (Rajendram et al. 2012).

Shortly after bevacizumab was approved, the same company Genentech developed another monoclonal antibody to VEGF-A, ranibizumab or Lucentis (Ferrara et al. 2006). Ranibizumab is a humanized mouse Fab fragment to an epitope of VEGF (AS82-91) from within the receptor binding domain (AS8-109) of VEGF165 and was approved by the FDA in June 2006 for treatment of AMD, June 2010 for treatment of macular edema from retinal vein occlusions (RVO), August 2012 for treatment of diabetic macular edema (DME), and February 2015 for the treatment of diabetic retinopathy. Like bevacizumab, ranibizumab has neutralizing activity on all VEGF isoforms. Prospective randomized controlled trials have proven the efficacy of ranibizumab in treating classic CNV in the ANCHOR trial (Brown et al. 2009), occult CNV in the MARINA trial (Rosenfeld et al. 2006), DME in RISE, RIDE, and RESTORE (Mitchell et al. 2011; Nguyen et al. 2012), BRVO in BRAVO (Campochiaro et al. 2010), and CRVO in CRUISE (Brown et al. 2010).

Several important differences exist between ranibizumab and bevacizumab: (1) ranibizumab (48 kDa) contains only the Fab fragment of the antibody, whereas bevacizumab (149 kDa) contains both the Fab and Fc fragments; (2) ranibizumab has six corresponding amino acids which differ from bevacizumab; (3) ranibizumab has one binding site for VEGF, whereas bevacizumab has two; (4) ranibizumab is produced in prokaryotic *Escherichia coli* and therefore is not glycosylated, whereas bevacizumab is produced in a eukaryotic cell line (CHO cells) and is N-glycosylated in its Fc region; and (5) ranibizumab costs over $2,000 per injection whereas bevacizumab costs approximately $60 per injection (Krispel et al. 2013).

However, it is unclear how much these differences between ranibizumab and bevacizumab translate into a clinically meaningful difference. The smaller size of ranibizumab was deliberate to enhance diffusion from the vitreous cavity into the retina and choroid (Ferrara et al. 2006). However, subsequent studies suggest that the predicted enhanced diffusion may not translate into a clinically meaningful therapeutic advantage (Martin et al. 2012). Affinity maturation of ranibizumab was also deliberate to increase the binding affinity to VEGF and increase the biologic activity compared to bevacizumab (Ferrara et al. 2006). While initial studies using a monovalent Fab-12 were significantly lower than what was later demonstrated for ranibizumab (Chen et al. 1999), later evaluation demonstrated that ranibizumab and bevacizumab had a similar affinity for VEGF-A165 given bevacizumab's bivalent

nature and dissociation constant (Papadopoulos et al. 2012). A possible disadvantage of the Fc fragment is that bevacizumab may be more stable systemically than is ranibizumab and thus have higher systemic levels (Miki et al. 2009). However, it is unclear whether this results in any clinically significant systemic effects.

Aflibercept (Eylea, Regeneron Pharmaceuticals Inc) is a recombinant fusion protein with the extracellular binding portions of VEGFR-1 and VEGFR-2 fused to the Fc portion of the human IgG1. Aflibercept received FDA approval in November 2011 for AMD, September 2012 for RVO, July 2014 for DME, and March 2015 for diabetic retinopathy. Aflibercept has been proven in large, randomized clinical trials for treating AMD in VIEW1 and 2 (Heier et al. 2012), CRVO in GALILEO (Korobelnik et al. 2014), BRVO in VIBRANT (Campochiaro et al. 2015), and DME in DA VINCI (Do et al. 2012).

Several studies have evaluated the relative efficacies of these bevacizumab and ranibizumab for treatment of AMD and found no difference in visual acuity or complications (Subramanian et al. 2010; Biswas et al. 2011; Chakravarthy et al. 2013; Kodjikian et al. 2013; Krebs et al. 2013). The CATT study found monthly treatments of bevacizumab not inferior to monthly ranibizumab, although ranibizumab had a greater mean decrease in central retinal thickness and bevacizumab had more serious systemic adverse events (CATT Research Group et al. 2011). A Cochrane Review including 12 randomized controlled trials including 5,496 patients for AMD found no difference in mean visual acuity outcomes between ranibizumab and bevacizumab, no difference in adverse events, and a statistically significant but clinically insignificant increase in reduction in central retinal thickness (−14 μm) with ranibizumab compared to bevacizumab (Solomon et al. 2014). Some disagreement also exists whether a difference in safety of bevacizumab and ranibizumab exists (Martin et al. 2012). Some retina specialists state that ranibizumab has been well studied in many randomized clinical trials with more long-term findings when compared with bevacizumab, although the superiority of ranibizumab over bevacizumab has not been proven. This remains a hot topic of debate among retina specialists given the significant difference in price between the medications, and both drugs are actively used to treat VEGF driven retinopathies currently.

A comparison of intravitreal bevacizumab, ranibizumab, and aflibercept for DME found that for visual acuity of 20/40 or better there was on average no difference in the improvement in visual acuity between the three treatments. For patients with visual acuity of 20/50 or worse, aflibercept resulted in more mean improvement in visual acuity (Diabetic Retinopathy Clinical Research Network et al. 2015).

Further modifications in VEGF therapy have occurred through the development of new anti-VEGF molecules. Conbercept (KH902; Chengdu Kanghong Biotech Co., Ltd., Sichuan, China) consists of the VEGF binding domains of the human VEGFR-1 and VEGFR-2 combined with the Fc portion of the human immunoglobulin G1. It binds the VEGF-A along with VEGF-B and placental growth factor (Wang et al. 2013). A randomized, double-masked, multicenter, phase 2 clinical

Table 2 Current non-VEGF targets in clinical trials

Drug	Drug target	Disease target	Clinicaltrials.gov ID	Phase	Number patients
Fractalkine (FKN)	CX3C chemokine	PDR	NCT00728598	1, completed 1998	30
OC-10X	Tubulin inhibitor	PDR, AMD	NCT01869933	1	10
Fovista (E10030)	Anti-PDGF-B	AMD	NCT02591914 NCT02214628 NCT01089517 NCT01944839	1 2 2 3,R	25 100 449 622
Palomid 529	TORC1/2 inhibitor of mTOR	AMD	NCT01033721 NCT01271270	1 1	13 5
AdGVPEDF.11D	PEDF	AMD	NCT00109499	1	
Sirolimus	mTOR IL-2	AMD	NCT00766337	2	62
Everolimus (RAD001)	mTOR	AMD	NCT00857259	2	16, Terminated
PF-04523655	Block RTP801 mTOR	DME AMD	NCT01445899 NCT00713518	2 2	258 152
ARC1905	Inhibit complement C5 aptamer	AMD	NCT00709527	1	60
iSONEP	S1P lipid	AMD	NCT01414153	2	158
ASP8232	Vascular adhesion protein-1 inhibitor	DME	NCT02302079	2	96
hI-con1	Factor VIIa inhibitor	AMD	NCT02358889	2	88
Tocilizumab	Antibody IL-6	DME	NCT02511067	2	66
iCo-007	inhibitor C-raf	DME	NCT01565148	2	208
Pf-04634817	CCR2/5 antagonist	DME	NCT01994291	2	212
ATG003 (mecamylamine)	Tubulin depolymerize, tight junction disruption	AMD	NCT00607750	2	60
RO6867461		DME AMD	NCT02699450 NCT01941082	2 1	150, R
Luminate (ALG-1001)	$\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin inhibitor	DME	NCT02348918	2	150

Apremilast (CC-10004)	Inhibit PDE4 (breaks down cAMP)	Behcet's	NCT00866359 NCT02307513	2 3	111 204
Efalizumab	Bind CD11a	DME	NCT00676559	1	0, Withdrawn
Zimura	Anti-C5 Aptamer	Polypoidal	NCT02397954	2	5
AKB-9778	Tie-2 activator	DME	NCT02050828	2	144
AL-78898A	Complement C3 inhibitor	AMD	NCT01157065	2	99
Volociximab	$\alpha_5\beta_1$ integrin antagonist	AMD	NCT00782093	1	63
JSM6427	$\alpha_5\beta_1$ integrin antagonist	AMD	NCT00536016	1	36

Abbreviations: *R* recruiting, *AMD* neovascular age-related macular degeneration, *DME* diabetic macular edema, *PDR* proliferative diabetic retinopathy, *RVO* retinal vein occlusion

trial of conbercept, AURORA, demonstrated the drug to be safe and efficacious with as needed dosing (Li et al. 2014a).

Recent concerns have been raised regarding possible increase in geographic atrophy with anti-VEGF administration, particularly ranibizumab (Grunwald et al. 2014, 2015). In addition, repeat administration of intravitreal anti-VEGF results in sustained elevation of intraocular pressure in some patients (Bakri et al. 2008; Pershing et al. 2013; Bakri et al. 2014; Dedania and Bakri 2015), suggesting the importance of VEGF to the trabecular meshwork.

10 Other Secreted Growth Factors Currently Under Study

In addition to VEGF, numerous other secreted growth factors that contribute to the angiogenesis cycle exist and are currently under investigation as potential therapeutic targets. Current anti-angiogenic non-VEGF mediated targets in clinical trials are summarized in Table 2. An important proangiogenic secreted molecule implicated in retinal neovascular disease is fibroblast derived growth factor (FGF) (Presta et al. 2005). FGF is a family that plays an important role in angiogenesis, wound healing, and embryonic development. FGF plays a role in the migration and proliferation of endothelial cells along with the proliferation of smooth muscle cells and fibroblasts. Basic FGF (bFGF), a secreted cytokine, regulates angiogenesis through induction of VEGF expression via the FGFR1/c-Src/p38/NF-kB (nuclear factor kB) signaling pathway, triggering angiogenesis of endothelial progenitor cells (Tzeng et al. 2015).

Platelet derived growth factor (PDGF) is critical for pericyte recruitment and maturation of neovascular vessels (Alvarez et al. 2006). Therapies targeting PDGF may destabilize neovascular tissue thereby enhancing current anti-VEGF approaches. This interplay among angiogenic factors is also observed between FGF and PDGF. FGF-2 works with PDGF-BB to upregulate expression of PDGFR-α and -β in newly formed blood vessels (Cao et al. 2003). Thus therapies targeting PDGF and FGF may prove to complement one another as well as current therapies targeting VEGF.

Placental-induced growth factor (PIGF) is a member of the VEGF family and plays an important role in several steps of vasculogenesis and angiogenesis through its receptor VEGFR-1 (Flt-1). An anti-Flt-1 antibody suppressed neovascularization in tumors and ischemic retina, and angiogenesis and inflammatory joint destruction in arthritis (Luttun et al. 2002). PIGF plays a role in endothelial cell migration and survival, recruitment of smooth muscle cells, and differentiation and activation of monocytes. PIGF mRNA expression was reduced by hypoxia in mice and elevated with anti-VEGF therapy (Zhou et al. 2014).

Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor and acts upon vascular endothelial cells to regulate cell growth, cell motility, and morphogenesis through epithelial mesenchymal interactions (Morishita et al. 2004). NK4, the N-terminal hairpin and subsequent four kringle domains of HGF, acts as the competitive antagonist for HGF and has been demonstrated to inhibit HGF-induced ERK1/2 (p44/42 mitogen-activated protein kinase) activation

to prevent angiogenesis and tumor growth in mice (Kuba et al. 2000). HGF has also been implicated in papillary thyroid cancer angiogenesis (Scarpino et al. 2003).

Pigment epithelium-derived factor (PEDF) plays an important role in endothelial cell migration and proliferation, apoptosis through the p38 MAPK pathway or FAS/FASL, and mediates angiogenesis through effects on VEGFR-1 and -2 (Longeras et al 2012). An N-terminal 34-amino acid peptide (PEDF-34) has been demonstrated to have anti-angiogenic properties along with anti-vasculogenic properties.

11 Pro-angiogenic Signaling Molecules as Therapeutic Targets

The VEGFR is a receptor tyrosine kinase and one of the earliest and most studied kinases targeted by synthetic small molecules for oncologic applications and may prove to be effective for the treatment of ocular neovascular disease. There are currently seven approved small molecules inhibitors targeting the VEGFR: sorafenib (Nexavar, Bayer), sunitinib (Sutent, Pfizer), pazopanib (Votrient, GlaxoSmithKline), axitinib (Inlyta, Pfizer), regorafenib (Stivarga, Bayer), nintedanib (Ofev, Boehringer Ingelheim), and lenvatinib (Lenvima, Eisai Inc.) (Wu et al. 2015). Both sorafenib and regorafenib are multitarget protein kinase inhibitors. Besides inhibition of VEGFR and B-raf, sorafenib has also been shown to be a potent low-nanomolar inhibitor of p38a (Simard et al. 2009). In addition, vandetanib (Caprelsa, AstraZeneca) is a multiple kinase inhibitor against EGFR, VEGFR, and RET (Knowles et al. 2006). Cabozantinib (Cometriq, Exelixis) is a dual MET and VEGFR2 inhibitor.

Numerous drugs targeting other signaling pathways that promote pathological angiogenesis have been developed or are being investigated for oncologic applications. Extension of these therapies for the treatment of ocular neovascular disease may follow. Trebananib (AMG 386) is a peptide-Fc fusion protein, or peptibody, reported to neutralize the interaction between angiopoietins (Ang1/2) and their Tie2 receptors, which has been shown to be promising in ovarian cancer (Marchetti et al. 2015). Nintedanib has activity against platelet derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) in addition to VEGFR, thus offering a broader spectrum of anti-angiogenic activity than simply VEGFR. Nintedanib has been shown to be effective in lung cancer (Hilberg et al. 2008; Reck et al. 2014). Linifanib (ABT-869) is a tyrosine kinase inhibitor that selectively targets VEGFR and PDGFR and has low off-target inhibitory activity and anti-angiogenic activity (Aversa et al. 2015).

The Wnt signaling pathways are a group of signal transduction pathways that pass extracellular signals through cell-surface receptors to intracellular. The canonical Wnt signaling pathway involves β-catenin. Wnt signaling pathways are activated by the binding of a Wnt-protein to a Frizzled receptor, which activates Dishevelled. Wnt controls cell fate specification, cell proliferation, and cell migration (Lad et al. 2009). A glial-derived extracellular ligand, Norrin, can stimulate

Wnt signaling and acts on the transmembrane receptor, Frizzled4, a coreceptor, Lrp5, and an auxiliary membrane protein, Tspan12, on the surface of developing endothelial cells. The resulting signal controls a transcriptional program that regulates endothelial growth and maturation (Ye et al. 2010). Therapies targeting Wnt signaling are also under development for the treatment of cancer; these, too, may prove effective as an anti-angiogenic approach for the treatment of ocular neovascular disease.

Nestin is an important molecule expressed in the cell soma of dividing neural progenitor cells and their leading processes. After this, it follows vascular branches, suggesting vasculogenesis along microglia migrating routes sustains its angiogenic potential (Lee et al. 2012). Endothelial nitric oxide synthases occur in the nuclei of endothelial vascular cells in vasoformative cells, suggesting that they may also play a role in vasculogenesis (McLeod et al. 2012). Neuropilins are transmembrane glycoproteins that are receptors for VEGF and are essential for blood vessel development and assist with the separation of veins and arteries (Fantin et al. 2011). In addition to VEGF, neuropilins bind to semaphorins, molecules critical for axon guidance which have recently been implicated also in retinal neovascular disease (Cerani et al. 2013).

Akt-mediated phosphorylation of Girdin, an actin-binding protein, promotes VEGF-dependent migration of endothelial cells and tube formation. Exogenously delivered adenovirus harboring Girdin short interfering RNA markedly inhibit VEGF-mediated angiogenesis (Kitamura et al. 2008). Mammalian target of rapamycin (mTOR), a key mediator of PI3K/Akt/mTOR signaling pathway, has recently emerged as a compelling molecular target in glioblastoma. The mTOR is a member of serine/threonine protein kinase family that functions as a central controller of growth, proliferation, metabolism, and angiogenesis, but its signaling is dysregulated in various human diseases especially in certain solid tumors including glioblastoma (Cui et al. 2015). mTOR has been implicated in the regulation of HIF-1 translation and may play a critical role in regulating HIF-directed angiogenesis in retinal neovascular disease.

The small GTPase RhoA and its downstream effectors, ROCK1 and ROCK2, are important mediators in a number of angiogenic processes, including EC migration, survival, and cell permeability, and suggest that Rho/ROCK inhibition may prove useful for the treatment of angiogenesis-related disorders (Bryan et al. 2010).

$\alpha_v\beta_3$ integrin has been reported as a promising therapeutic target for angiogenesis. GOPPP, a novel antagonist of $\alpha_v\beta_3$ integrin, has been shown to inhibit the pro-angiogenic effects of vitronectin on HUVECs, including adhesion, proliferation, and migration, and inhibit ERK1/2 and Akt phosphorylation. HIF-1α and VEGF were also inhibited by GOPPP in mice (Li et al. 2014b). Proliferative diabetic retinopathy has been shown to recruit bone-marrow-derived CD133+ endothelial progenitor cells and CD14+ monocytes to assist (Abu El-Asrar et al. 2011). Other integrins under active investigation include $\alpha_v\beta_5$ and $\alpha_5\beta_1$ in current clinical trials for AMD and diabetic macular edema.

MicroRNAs (miRNA) are small non-coding RNA molecules (containing approximately 22 nucleotides) that function in RNA silencing and post-transcriptional regulation of gene expression. A single miRNA can regulate the

Table 3 Future anti-angiogenic targets to consider

Drug target	Relevant literature/studies
HIF-1/2	Subhani et al. (2016)
Angiopoietin-like 4 (ANGPTL4)	Babapoor-Farrokhran et al. (2015), Xin et al. (2013), and Kwon et al. (2015)
Stromal-derived factor-1 (SDF-1) and its receptor CXCR4	Ghanem et al. (2014)
Metabolic gene products	Treps et al. (2016)
Matrix metalloproteinases (MMP)	Sampieri et al. (2013), Zhang et al. (2016), and Chang et al. (2016)
Inflammation and inflammatory cytokines	de Oliveira Dias et al. (2011) and Schor and Schor (2010)
Reactive oxygen species (ROS)	Wilkinson-Berka et al. (2013)
Stem cell and endothelial progenitor therapy	Nazari et al. (2015)
Integrin antagonists	Salehi-Had et al. (2011), Varner et al. (1996), and Tolentino (2009)
Methionine aminopeptidase	Ma et al. (2011) and Mauriz et al. (2010)

expression of hundreds of gene products and can have broad effects on cell function. MiRNA-132 is a highly upregulated miRNA in a human embryonic stem cell model of vasculogenesis and was found to be highly expressed in the endothelium of human tumors. Anti-miRNA-132 has been shown to effectively inhibit neovascularization (Anand et al. 2010). However, whether therapies targeting microRNAs will be specific enough for the treatment of human disease remains unknown.

12 Emerging Therapeutic Targets

Future anti-angiogenic approaches may include therapies upstream of the angiogenic secreted factors [e.g., HIF-1 (Subhani et al. 2016)] or novel angiogenic secreted molecules [e.g., angiopoietin-like 4, or ANGPTL4 (Xin et al. 2013; Sodhi and Montaner 2015)] or signaling pathways regulated by these molecules. However, recent focus has been directed at the molecules that regulate other steps in pathological angiogenesis (Table 3). Appreciation for the role of circulating progenitor cells and hematopoietic stem cells from the bone marrow to pathological angiogenesis (Liekens et al. 2010) has exposed the SDF1/CXCR4 axis as a novel therapeutic target for ocular neovascular disease. The extracellular matrix plays an essential role in retinal neovascularization. MMPs are necessary to degrade the basement membrane to allow migrating vascular endothelial cells to escape from the parent vessel (Rodrigues et al. 2013). Although therapies targeting MMPs have not proven successful, other molecules that regulate extracellular matrix protein production have emerged as potential targets including transforming growth factor (TGF)-α and -β, endoglin, epidermal growth factor (EGF), inflammatory cytokines

including IL-6 and 8, TNF-α and β inhibitors, α5β1 integrin receptor inhibitor, and $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Another critical step in pathological angiogenesis is the promotion of endothelial cell survival through both intrinsic (e.g., VEGF) and extrinsic mechanisms. The latter is ensured through stabilization of endothelial cells through recruitment of pericytes, smooth muscle cells, and deposition of extracellular matrix proteins in addition to focal adhesion kinase (FAK) (Roy-Luzarraga and Hodivala-Dilke 2016). In addition, endothelial cell transdifferentiation has exposed to additional targets including ID1 and ID3 (Ruzinova et al. 2003).

13 Vascular Permeability Targets

There is significant overlap between pathological angiogenesis (and retinal neovascularization) and vascular hyperpermeability (and macular edema). Indeed, one of the first steps in pathological angiogenesis is the induction of vascular permeability. It is therefore not surprising that many of the therapeutic targets for retinal neovascularization have proven (or may prove) effective for the treatment of macular edema. This is most notable for therapies targeting VEGF. Nonetheless, macular edema is a major cause of vision loss in numerous disorders, including uveitis, pars planitis, post-operative, and retinal degenerations such as retinitis pigmentosa, in which retinal neovascularization is less common, and there are several mediators that promote vascular permeability independent of their ability to promote angiogenesis.

Macular edema results from a breakdown of the blood–retinal barrier (BRB), which is composed of an inner and outer component (Cunha-Vaz 1976). The inner component is formed by tight functions and adherens junction between vascular endothelial cells. Pericytes and perivascular astrocytes mediate the inner component also and the paucity of intraendothelial cell vesicles present under normal conditions. The outer component is established by tight junctions between RPE cells that prevent fluid from choroidal vessels entering the retina (Vinores 1995; Rizzolo 1997; Vinores et al. 1999).

In diabetes, an upregulation of trans-endothelial vesicular transport and increased membrane permeability correlates with BRB breakdown. The players in vascular permeability are identical to those in pathological angiogenesis, including retinal glial cells, vascular endothelial cells, and neutrophils, which together promote breakdown of adherens and tight junctions and increase vesicular transport. And the secreted factors observed in neovascularization, including VEGF, TNF-α, and IL-1β, are elevated also in macular edema (Cuff et al. 1996; Luna et al. 1997). Similarly, ANGPTL4, an emerging target in pathological angiogenesis and retinal neovascularization (Babapoor-Farrokhran et al. 2015) may also play an important role in vascular permeability and macular edema (Xin et al. 2013). The downstream signaling molecules are also similar between retinal neovascularization and diabetic macular edema. As such, src kinase inhibitors (Doukas et al. 2008) and tyrosine kinase inhibitors such as pazopanib have been shown to reduce diabetic macular edema in animal models (Thakur et al. 2011).

Protein kinase C (PKC) inhibitors such as ruboxistaurin showed initial promise in reducing diabetic macular edema (Gálvez 2011; Aiello et al. 2011), although some phase 3 studies have shown mixed results (Sheetz et al. 2013).

14 Molecular Imaging and Theranostics

Currently available ophthalmic imaging modalities are capable of imaging retinal pathologic anatomic changes, including hemorrhages, exudates, and edema, with unprecedented resolution. However, anatomical abnormalities are the end-product of complex molecular processes. Subclinical molecular changes occur before retinal disease can be detected by current anatomy-driven imaging instruments. Thus, the field of molecular imaging is emerging as an important factor to assist with early disease detection, improved treatment monitoring, and improved understanding of retinal pathophysiology (Xie et al. 2012; Capozzi et al. 2013; Evans et al. 2014). Several recent studies have reported feasibility of molecular imaging in detecting retinal ganglion cells (RGCs), RPE cells, vascular endothelial cells, VEGFR, and leukocytes.

Detection of apoptosing retinal cells (DARC) has been described for the single-cell detection of RGC apoptosis (Cordeiro et al. 2004; Coxon et al. 2011). In DARC, Annexin V was intravitreally injected to specifically bind the apoptosis biomarker phosphatidylserine (PS), and then it was detected by ophthalmic fluorescence imaging instrumentation. A peptide-based fluorescent probe (TcapQ) sensitive to active caspases such as caspase 3 involved in RGC apoptosis has also been described in vivo to quantify apoptotic RGCs (Barnett et al. 2009). Another promising opportunity for molecular imaging of RGCs lies in imaging RGC dysfunction before cell death. Several imaging probe such as reactive oxygen (ROS) (Dickinson et al. 2011), mitochondria permeability transition pore (Vrabec et al. 2003), and E glutamate (Okubo et al. 2010) hold great promise in improving the molecular imaging of RGCs. RPE-related molecular targets that have been evaluated include ROS (King et al. 2004), β-amyloid, esterified cholesterol, and carbohydrate moieties in drusen (Hageman et al. 2001).

Vascular endothelial cells have emerged as critical surface biomarkers of neovascularization and as potential candidates for development of targeted contrast agents for ophthalmic imaging of retinal and choroidal neovascularization. The C-C chemokine receptor 3 (CCR3) is a promising biomarker of choroidal neovascularization (CNV), as demonstrated by CCR3 expression on choroidal neovascular endothelial cells in human CNV specimens (Takeda et al. 2009). Other promising biomarkers include targeting proliferating endothelial, endoglin (Grisanti et al. 2004), and integrin $\alpha_v\beta_3$ (Li et al. 2010; Peiris et al. 2012). VEGFR-2 expression has been demonstrated with molecular imaging to be elevated in retinal capillaries in diabetes (Sun et al. 2014). OCT has been used with anti-mouse CD45 coated gold nanorods to visualize leukocytes at sites of laser-induced retinal injury (Sen et al. 2016). Photoacoustic imaging can also be used to perform molecular imaging (de la Zerda et al. 2010; Hu et al. 2015). Molecular imaging is

now further evolving into the development of theranostics, agents that combine diagnostic imaging with targeted therapy (Ding et al. 2013; Yan et al. 2016). While currently in its infancy, molecular imaging of the retina is rapidly developing and will play a critical role in early disease diagnosis and treatment monitoring in the near future.

15 Drug Delivery Pathways

Treatment with anti-VEGF is often transient and must be repeated with rapid regrowth of neovascularization on cessation of treatment. In addition to intravitreal injections which are the most common routes of administration, numerous additional pathways of delivery are being investigated. Recent developments in drug delivery have led to investigations on longer-lasting agents through biodegradable implants, gene delivery, biodegradable polymers, suprachoroidal delivery, microspheres, contact lenses with sustained delivery, punctal plugs with sustained delivery, nanoparticles, liposomes, and gels (Querques et al. 2015). ForSight Vision has developed a refillable, nonbiodegradable port delivery system that is surgically implanted in the pars plana beneath the conjunctiva through a 3.2 mm scleral incision. A phase 1 study was conducted in Latvia included 20 treatment-naïve patients with neovascular AMD (Rubio 2014). The primary endpoint of the study was at 1 year. There were four serious adverse effects, including one case of endophthalmitis, two cases of persistent vitreous hemorrhage, and one case of traumatic cataract. The visual acuity gain for all patients at month 12 was 10 letters, and the average number of refills for the full 20-patient cohort was 4.8. A phase 2 study is being planned.

Topical therapy is also being developed, pazopanib (GlaxoSmithKline, Brentford, UK), a multi-tyrosine kinase inhibitor having an effect on VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β. A study evaluating 70 patients with AMD treated for 28 days found a decrease in central retinal thickness and an increase in visual acuity in only a subset of patients (Danis et al. 2014). Bevacizumab therapy was initially investigated in AMD as a systemic treatment given intravenously, and oral therapies are also being investigated.

Another strategy of sustained delivery is through gene delivery through adeno-associated viral vectors (AAV). Gene therapy trials have been completed for Leber's congenital amaurosis and have shown safety and promising results (Maguire et al. 2008). AAV2-sFLT01 (Genzyme, Cambridge, MA, USA) allows delivery of VEGFR1 (sFlt1) and has demonstrated sustained expression in animals (Pechan et al. 2009; Lukason et al. 2011). Similarly, AVA-101 (rAAV.sFLT-1 recombinant adeno-associated virus) is currently undergoing a phase I study (ClinicalTrials.gov identifier: NCT01494805).

Additional routes of administration which can be considered include transscleral, subretinal, and high-velocity (needleless) delivery (Todorich et al. 2014), siRNA, and cell-based therapy. Single chain antibody fragments are being developed which allow for sustained delivery due to their small size. A phase

I study of the safety and tolerability of a single chain antibody fragment ESBA1008 (Alcon, Fort Worth, Tex., USA) versus ranibizumab has been completed in 194 patients in a prospective, randomized, multicenter trial. Encapsulated cell technology involves implants which can continuously produce recombinant therapy without needing to be refilled. NT-503 (Neurotech Pharmaceuticals, RI, USA), an intraocular implant delivering anti-VEGF, is being tested in a phase I prospective, multicenter study (Querques et al. 2015). In addition to pharmacologic therapies, laser photocoagulation can be used along with photodynamic therapy, transpupillary thermotherapy, and radiation therapy as a possible treatment by itself or in conjunction with pharmacotherapy.

16 Conclusion

Anti-angiogenic pharmacologic therapy has become an indispensable component in the management of diseases of the vitreous, choroid, and retina. Anti-angiogenic pharmacotherapy has revolutionized the treatment of many ocular conditions, including diabetic retinopathy, retinal vein occlusions, pathologic myopia, choroidal neovascularization, and age-related macular degeneration. Our improving understanding of the pathophysiology of embryology, angiogenesis, and vasculogenesis has allowed us to increasingly target a select tissue while minimizing the side effects of therapy. Selective therapeutic approaches will achieve desirable biochemical, cellular, and tissue effects while minimizing unwanted damage, and will improve our understanding of the function and pathology of posterior segment diseases. Continuous innovations in pharmacotherapy and progress in understanding of retinal pathophysiology make us believe that improvements in the treatment of retinal diseases using anti-angiogenic therapy will continue for many years to come.

Conflict of Interest Johns Hopkins has filed a patent application on the use of technology to modulate the levels of ANGPTL4 for the treatment of ocular neovascular disease (US patent 14/394, 152). This work was supported by the National Eye Institute, National Institutes of Health Grant, K08-EY021189 (AS) and an Unrestricted Grant from Research to Prevent Blindness (AS), and the Heed Ophthalmic Foundation Fellows Grant (YMP) and the National Eye Institute, National Institutes of Health Grant, K12-EY022299-4 (YMP). Dr. Sodhi gratefully acknowledges the support he receives as a Special Scholar Award recipient from Research to Prevent Blindness, Inc. The funding organizations had no role in the design or conduct of this research.

References

Abu El-Asrar AM, Struyf S, Verbeke H, Van Damme J, Geboes K (2011) Circulating bone-marrow-derived endothelial precursor cells contribute to neovascularization in diabetic epiretinal membranes. Acta Ophthalmol 89(3):222–228

Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, Yeo KT (1994) Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 118(4):445–450

Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE et al (1994) Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331(22):1480–1487

Aiello LP, Vignati L, Sheetz MJ, Zhi X, Girach A, Davis MD, Wolka AM, Shahri N, Milton RC, PKC-DRS and PKC-DRS2 Study Groups (2011) Oral protein kinase c β inhibition using ruboxistaurin: efficacy, safety, and causes of vision loss among 813 patients (1,392 eyes) with diabetic retinopathy in the protein kinase C β inhibitor-diabetic retinopathy study and the protein kinase C β inhibitor-diabetic retinopathy study 2. Retina 31(10):2084–2094

Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E (1995) Vascular endothelial growth factor acts as a survival factor for new formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1:1024–1028

Alvarez RH, Kantarjian HM, Cortes JE (2006) Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin Proc 81:1241–1257

Amin RH, Frank RN, Kennedy A, Eliott D, Puklin JE, Abrams GW (1997) Vascular endothelial growth factor is present in glial cells of the retina and optic nerve of human subjects with nonproliferative diabetic retinopathy. Invest Ophthalmol Vis Sci 38(1):36–47

Anand S, Majeti BK, Acevedo LM, Murphy EA, Mukthavaram R, Scheppke L, Huang M, Shields DJ, Lindquist JN, Lapinski PE, King PD, Weis SM, Cheresh DA (2010) MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. Nat Med 16(8):909–914

Ashton N (1970) Retinal angiogenesis in the human embryo. Br Med Bull 26:103–106

Aversa C, Leone F, Zucchini G, Serini G, Geuna E, Milani A, Valdembri D, Martinello R, Montemurro F (2015) Linifanib: current status and future potential in cancer therapy. Expert Rev Anticancer Ther 15(6):677–687

Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ (2006) Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 113 (3):363–372

Babapoor-Farrokhran S, Jee K, Puchner B, Hassan SJ, Xin X, Rodrigues M, Kashiwabuchi F, Ma T, Hu K, Deshpande M, Daoud Y, Solomon S, Wenick A, Lutty GA, Semenza GL, Montaner S, Sodhi A (2015) Angiopoietin-like 4 is a potent angiogenic factor and a novel therapeutic target for patients with proliferative diabetic retinopathy. Proc Natl Acad Sci U S A 112(23):E3030–E3039

Bakri SJ, McCannel CA, Edwards AO, Moshfeghi DM (2008) Persistent ocular hypertension following intravitreal ranibizumab. Graefes Arch Clin Exp Ophthalmol 246:955–958

Bakri SJ, Moshfeghi DM, Francom S, Rundle AC, Reshef DS, Lee PP, Schaeffer C, Rubio RG, Lai P (2014) Intraocular pressure in eyes receiving monthly ranibizumab in 2 pivotal age-related macular degeneration clinical trials. Ophthalmology 121:1102–1108

Barnett EM, Zhang X, Maxwell D, Chang Q, Piwnica-Worms D (2009) Single-cell imaging of retinal ganglion cell apoptosis with a cell-penetrating, activatable peptide probe in an in vivo glaucoma model. Proc Natl Acad Sci U S A 106:9391–9396

Beach JM, Schwenzer KJ, Srinivas S, Kim D, Tiedeman JS (1999) Oximetry of retinal vessels by dual-wavelength imaging: calibration and influence of pigmentation. J Appl Physiol 86 (2):748–758

Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A, Delbono O (2014) Type-2 pericytes participate in normal and tumoral angiogenesis. Am J Physiol Cell Physiol 307 (1):C25–C38

Biswas P, Sengupta S, Choudhary R, Home S, Paul A, Sinha S (2011) Comparative role of intravitreal ranibizumab versus bevacizumab in choroidal neovascular membrane in age-related macular degeneration. Indian J Ophthalmol 59(3):191–196

Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T, ANCHOR Study Group (2009) Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: two-year results of the ANCHOR study. Ophthalmology 116(1):57–65

Brown DM, Campochiaro PA, Singh RP, Li Z, Gray S, Saroj N, Rundle AC, Rubio RG, Murahashi WY, CRUISE Investigators (2010) Ranibizumab for macular edema after central retinal vein occlusion: six-month primary end point results of a phase III study. Ophthalmology 117:1124–1133

Bryan BA, Dennstedt E, Mitchell DC, Walshe TE, Noma K, Loureiro R, Saint-Geniez M, Campaigniac JP, Liao JK, D'Amore PA (2010) RhoA/ROCK signaling is essential for multiple aspects of VEGF-mediated angiogenesis. FASEB J 24(9):3186–3195

Burri PH, Hlushchuk R, Djonov V (2004) Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. Dev Dyn 231(3):474–488

Campochiaro PA, Heier JS, Feiner L, Gray S, Saroj N, Rundle AC, Murahashi WY, Rubio RG, BRAVO Investigators (2010) Ranibizumab for macular edema after branch retinal vein occlusion: six-month primary end point results of a phase III study. Ophthalmology 117:1102–1112

Campochiaro PA, Clark WL, Boyer DS, Heier JS, Brown DM, Vitti R, Kazmi H, Berliner AJ, Erickson K, Chu KW, Soo Y, Cheng Y, Haller JA (2015) Intravitreal aflibercept for macular edema following branch retinal vein occlusion: the 24-week results of the VIBRANT study. Ophthalmology 122(3):538–544

Cao R, Brakenhielm E, Pawliuk R, Wariaro D, Post MJ, Wahlberg E, Leboulch P, Cao Y (2003) Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. Nat Med 9(5):604–613

Capozzi ME, Gordon AY, Penn JS, Jayagopal A (2013) Molecular imaging of retinal disease. J Ocul Pharmacol Ther 29(2):275–286

Caprara C, Thiersch M, Lange C, Joly S, Samardzija M, Grimm C (2011) HIF1A is essential for the development of the intermediate plexus of the retinal vasculature. Invest Ophthalmol Vis Sci 52:2109–2117

CATT Research Group, Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ (2011) Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med 364(20):1897–1908

Cerani A, Tetreault N, Menard C, Lapalme E, Patel C, Sitaras N, Beaudoin F, Leboeuf D, De Guire V, Binet F, Dejda A, Rezende FA, Miloudi K, Sapieha P (2013) Neuron-derived semaphorin 3A is an early inducer of vascular permeability in diabetic retinopathy via neuropilin-1. Cell Metab 18(4):505–518

Chakravarthy U, Adamis AP, Cunningham ET Jr, Goldbaum M, Guyer DR, Katz B, Patel M, VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group (2006) Year 2 efficacy results of 2 randomized controlled clinical trials of pegaptanib for neovascular age-related macular degeneration. Ophthalmology 113(9):1508.e1–25

Chakravarthy U, Harding SP, Rogers CA, Downes SM, Lotery AJ, Culliford LA, Reeves BC, IVAN study investigators (2013) Alternative treatments to inhibit VEGF in age-related choroidal neovascularisation: 2-year findings of the IVAN randomised controlled trial. Lancet 382 (9900):1258–1267

Chang JH et al (2016) Matrix metalloproteinase 14 modulates signal transduction and angiogenesis in the cornea. Surv Ophthalmol 61(4):478–497

Chan-Ling T, Gock B, Stone J (1995) The effect of oxygen on vasoformative cell division. Evidence that 'physiologic hypoxia' is the stimulus for normal retinal vasculogenesis. Invest Ophthalmol Vis Sci 36:1201–1214

Chan-Ling T, Mcleod DS, Hughes S, Bax-ter L, Chu Y, Hasegawa T, Lutty GA (2004) Astrocyte-endothelial cell relation-ships during human retinal vascular development. Invest Ophthalmol Vis Sci 45:2020–2032

Chen Y, Wiesmann C, Fuh G, Li B, Christinger HW, McKay P, de Vos AM, Lowman HB (1999) Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity matured Fab in complex with antigen. J Mol Biol 293:865–881

Chu Y, Hughes S, Chan-Ling T (2001) Differentiation and migration of astrocyte precursor cells and astrocytes in human fetal retina: relevance to optic nerve coloboma. FASEB J 15:2013–2015

Claxton S, Fruttiger M (2003) Role of arteries in oxygen induced vaso-obliteration. Exp Eye Res 77:305–311

Cohen MH, Gootenberg J, Keegan P, Pazdur R (2007) FDA drug approval summary: bevacizumab plus FOLFOX4 as second-line treatment of colorectal cancer. Oncologist 12(3):356–361

Cordeiro MF, Guo L, Luong V, Harding G, Wang W, Jones HE, Moss SE, Sillito AM, Fitzke FW (2004) Real-time imaging of single nerve cell apoptosis in retinal neurodegeneration. Proc Natl Acad Sci U S A 101:13352–13356

Coxon KM, Duggan J, Cordeiro MF, Moss SE (2011) Purification of annexin V and its use in the detection of apoptotic cells. Methods Mol Biol 731:293–308

Cuff CA, Martiney JA, Berman JW, Brosnan CF (1996) Differential effects of transforming growth factor-ß-1 on interleukin-1-induced cellular inflammation and vascular permeability in the rabbit retina. J Neuroimmunol 70:21–28

Cui YH, Chen J, Xu T, Tian HL (2015) Structure-based grafting and identification of kinase-inhibitors to target mTOR signaling pathway as potential therapeutics for glioblastoma. Comput Biol Chem 54:57–65

Cunha-Vaz JG (1976) The blood-retinal barriers. Doc Ophthalmol 41:287–327

D'Amico DJ, Masonson HN, Patel M, Adamis AP, Cunningham ET Jr, Guyer DR, Katz B, VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group (2006) Pegaptanib sodium for neovascular age-related macular degeneration: two-year safety results of the two prospective, multicenter, controlled clinical trials. Ophthalmology 113(6):992–1001

Danis R, McLaughlin MM, Tolentino M, Staurenghi G, Ye L, Xu CF, Kim RY, Johnson MW, Group PEDS (2014) Pazopanib eye drops: a randomised trial in neovascular age-related macular degeneration. Br J Ophthalmol 98:172–178

Datta M, Via LE, Kamoun WS, Liu C, Chen W, Seano G, Weiner DM, Schimel D, England K, Martin JD, Gao X, Xu L, Barry CE 3rd, Jain RK (2015) Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery. Proc Natl Acad Sci U S A 112(6):1827–1832

Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP (1999) Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science 285(5425):245–248

de la Zerda A, Paulus YM, Teed R, Bodapati S, Dollberg Y, Khuri-Yakub BT, Blumenkranz MS, Moshfeghi DM, Gambhir SS (2010) Photoacoustic ocular imaging. Opt Lett 35(3):270–272

de Oliveira Dias JR, Rodrigues EB, Maia M, Magalhães O Jr, Penha FM, Farah ME (2011) Cytokines in neovascular age-related macular degeneration: fundamentals of targeted combination therapy. Br J Ophthalmol 95(12):1631–1637

Dedania VS, Bakri SJ (2015) Sustained elevation of intraocular pressure after intravitreal anti-VEGF agents: what is the evidence? Retina 35(5):841–858

Diabetic Retinopathy Clinical Research Network, Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, Arnold-Bush B, Baker CW, Bressler NM, Browning DJ, Elman MJ, Ferris FL, Friedman SM, Melia M, Pieramici DJ, Sun JK, Beck RW (2015) Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. N Engl J Med 372(13):1193–1203

Dickinson BC, Tang Y, Chang Z, Chang CJ (2011) A nuclear-localized fluorescent hydrogen peroxide probe for monitoring sirtuin-mediated oxidative stress responses in vivo. Chem Biol 18:943–948

Ding Y, Li S, Nie G (2013) Nanotechnological strategies for therapeutic targeting of tumor vasculature. Nanomedicine (Lond) 8(7):1209–1222

DiPietro LA (2016) Angiogenesis and wound repair: when enough is enough. J Leukoc Biol. pii: jlb.4MR0316-102R [Epub ahead of print]

Djonov VG, Kurz H, Burri PH (2002) Optimality in the developing vascular system: branching remodeling by means of intussusception as an efficient adaptation mechanism. Dev Dyn 224 (4):391–402

Do DV, Nguyen QD, Boyer D, Schmidt-Erfurth U, Brown DM, Vitti R, Berliner AJ, Gao B, Zeitz O, Ruckert R, Schmelter T, Sandbrink R, Heier JS, da Vinci Study Group (2012) One-year outcomes of the da Vinci Study of VEGF Trap-Eye in eyes with diabetic macular edema. Ophthalmology 119(8):1658–1665

Doukas J, Mahesh S, Umeda N, Kachi S, Akiyama H, Yokoi K, Cao J, Chen Z, Dellamary L, Tam B, Racanelli-Layton A, Hood J, Martin M, Noronha G, Soll R, Campochiaro PA (2008) Topical administration of a multi-targeted kinase inhibitor suppresses choroidal neovascularization and retinal edema. J Cell Physiol 216(1):29–37

Early Treatment Diabetic Retinopathy Study Research Group (1991) Results from the early treatment diabetic retinopathy study. Ophthalmology 98(Suppl 5):739–840

Epstein DL, Algvere PV, von Wendt G, Seregard S, Kvanta A (2012) Bevacizumab for macular edema in central retinal vein occlusion: a prospective, randomized, double-masked clinical study. Ophthalmology 119(6):1184–1189

Evans SM, Kim K, Moore CE, Uddin MI, Capozzi ME, Craft JR, Sulikowski GA, Jayagopal A (2014) Molecular probes for imaging of hypoxia in the retina. Bioconjug Chem 25 (11):2030–2037

Fantin A, Schwarz Q, Davidson K, Normando EM, Denti L, Ruhrberg C (2011) The cytoplasmic domain of neuropilin 1 is dispensable for angiogenesis, but promotes the spatial separation of retinal arteries and veins. Development 138(19):4185–4191

Favier B, Alam A, Barron P, Bonnin J, Laboudie P, Fons P, Mandron M, Herault JP, Neufeld G, Savi P, Herbert JM, Bono F (2006) Neuropilin-2 interacts with VEGFR-2 and VEGFR-3 and promotes human endothelial cell survival and migration. Blood 108(4):1243–1250

Feeney SA, Simpson DA, Gardiner TA, Boyle C, Jamison P, Stitt AW (2003) Role of vascular endothelial growth factor and placental growth factors during retinal vascular development and hyaloid regression. Invest Ophthalmol Vis Sci 44(2):839–847

Ferrara N (2005) VEGF as a therapeutic target in cancer. Oncology 69(Suppl 3):11–16

Ferrara N, Damico L, Shams N, Lowman H, Kim R (2006) Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. Retina 26:859–870

Figg W, Folkman J (eds) (2008) Angiogenesis: an integrative approach from science to medicine. Springer, New York

Gálvez MI (2011) Protein kinase C inhibitors in the treatment of diabetic retinopathy. Review. Curr Pharm Biotechnol 12(3):386–391

Gariano RF (2003) Cellular mechanisms in retinal vascular development. Prog Retin Eye Res 22:295–306

Gariano RF, Gardner TW (2005) Retinal angiogenesis in development and disease. Nature 438:960–966

Gariano RF, Hu D, Helms J (2006) Expression of angiogenesis-related genes during retinal development. Gene Expr Patterns 6(2):187–192

Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C (2003) VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 161(6):1163–1177

Ghanem I, Riveiro ME, Paradis V, Faivre S, de Parga PM, Raymond E (2014) Insights on the CXCL12-CXCR4 axis in hepatocellular carcinoma carcinogenesis. Am J Transl Res 6 (4):340–352

Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR, VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group (2004) Pegaptanib for neovascular age-related macular degeneration. N Engl J Med 351(27):2805–2816

Grisanti S, Canbek S, Kaiserling E, Adam A, Lafaut B, Gelisken F, Szurman P, Henke-Fahle S, Oficjalska-Mlynczak J, Bartz-Schmidt KU (2004) Expression of endoglin in choroidal neovascularization. Exp Eye Res 78:207–213

Grunwald JE, Daniel E, Huang J, Ying GS, Maguire MG, Toth CA, Jaffe GJ, Fine SL, Blodi B, Klein ML, Martin AA, Hagstrom SA, Martin DF, CATT Research Group (2014) Risk of

geographic atrophy in the comparison of age-related macular degeneration treatments trials. Ophthalmology 121(1):150–161

Grunwald JE, Pistilli M, Ying GS, Maguire MG, Daniel E, Martin DF, Comparison of Age-related Macular Degeneration Treatments Trials Research Group (2015) Growth of geographic atrophy in the comparison of age-related macular degeneration treatments trials. Ophthalmology 122(4):809–816

Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 20:705–732

Heier JS, Brown DM, Chong V, Korobelnik JF, Kaiser PK, Nguyen QD, Kirchhof B, Ho A, Ogura Y, Yancopoulos GD, Stahl N, Vitti R, Berliner AJ, Soo Y, Anderesi M, Groetzbach G, Sommerauer B, Sandbrink R, Simader C, Schmidt-Erfurth U, VIEW 1 and VIEW 2 Study Groups (2012) Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology 119(12):2537–2548

Hilberg F, Roth GJ, Krssak M, Kautschitsch S, Sommergruber W, Tontsch-Grunt U, Garin-Chesa P, Bader G, Zoephel A, Quant J, Heckel A, Rettig WJ (2008) BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. Cancer Res 68:4774–4782

Hu Z, Wang X, Liu Q, Paulus YM (2015) Photoacoustic imaging in ophthalmology. Int J Ophthalmol Eye Sci 3(8):126–132

Hubbi ME, Gilkes DM, Hu H, Kshitiz, Ahmed I, Semenza GL (2014) Cyclin-dependent kinases regulate lysosomal degradation of hypoxia-inducible factor 1α to promote cell-cycle progression. Proc Natl Acad Sci U S A 111(32):E3325–E3334

Hughes S, Yang H, Chan-Ling T (2000) Vascularization of the human fetal retina: roles of vasculogenesis and angiogenesis. Invest Ophthalmol Vis Sci 41:1217–1228

Jiang A, Zhang M, Liu Z (2005) Angioblasts in adult and its role in ocular disorders due to neovascularization. Yan Ke Xue Bao 21(3):158–162, 178

Kaelin WG (2005) Proline hydroxylation and gene expression. Annu Rev Biochem 74:115–128

Kapany NS, Peppers NA, Zweng HC, Flocks M (1963) Retinal photocoagulation by lasers. Nature 199:146–149

Kaur C, Foulds WS, Ling EA (2008) Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. Prog Retin Eye Res 27(6):622–647

King A, Gottlieb E, Brooks DG, Murphy MP, Dunaief JL (2004) Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells. Photochem Photobiol 79:470–475

Kitamura T, Asai N, Enomoto A, Maeda K, Kato T, Ishida M, Jiang P, Watanabe T, Usukura J, Kondo T, Costantini F, Murohara T, Takahashi M (2008) Regulation of VEGF-mediated angiogenesis by the Akt/PKB substrate Girdin. Nat Cell Biol 10(3):329–337

Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, Ibáñez CF, McDonald NQ (2006) Structure and chemical inhibition of the RET tyrosine kinase domain. J Biol Chem 281:33577–33587

Kodjikian L, Souied EH, Mimoun G, Mauget-Faÿsse M, Behar-Cohen F, Decullier E, Huot L, Aulagner G, GEFAL Study Group (2013) Ranibizumab versus bevacizumab for neovascular age-related macular degeneration: results from the GEFAL noninferiority randomized trial. Ophthalmology 120(11):2300–2309

Korobelnik JF, Holz FG, Roider J, Ogura Y, Simader C, Schmidt-Erfurth U, Lorenz K, Honda M, Vitti R, Berliner AJ, Hiemeyer F, Stemper B, Zeitz O, Sandbrink R, GALILEO Study Group (2014) Intravitreal aflibercept injection for macular edema resulting from central retinal vein occlusion: one-year results of the phase 3 GALILEO study. Ophthalmology 121(1):202–208

Krady JK, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, Levison SW (2005) Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. Diabetes 54(5):1559–1565

Krebs I, Schmetterer L, Boltz A, Told R, Vécsei-Marlovits V, Egger S, Schönherr U, Haas A, Ansari-Shahrezaei S, Binder S, MANTA Research Group (2013) A randomised double-masked trial comparing the visual outcome after treatment with ranibizumab or bevacizumab in patients with neovascular age-related macular degeneration. Br J Ophthalmol 97 (3):266–271

Krispel C, Rodrigues M, Xin X, Sodhi A (2013) Ranibizumab in diabetic macular edema. World J Diabetes 4(6):310–318

Kuba K, Matsumoto K, Date K, Shimura H, Tanaka M, Nakamura T (2000) HGF/NK4, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppresses tumor growth and metastasis in mice. Cancer Res 60(23):6737–6743

Kurihara T, Westenskow PD, Friedlander M (2014) Hypoxia-inducible factor (HIF)/vascular endothelial growth factor (VEGF) signaling in the retina. Adv Exp Med Biol 801:275–281

Kwon SH, Shin JP, Kim IT, Park DH (2015) Aqueous levels of angiopoietin-like 4 and semaphorin 3E correlate with nonperfusion area and macular volume in diabetic retinopathy. Ophthalmology 122(5):968–975

Lad EM, Cheshier SH, Kalani MY (2009) Wnt-signaling in retinal development and disease. Stem Cells Dev 18(1):7–16

Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 16(12):1466–1471

Lee JH, Park HS, Shin JM, Chun MH, Oh SJ (2012) Nestin expressing progenitor cells during establishment of the neural retina and its vasculature. Anat Cell Biol 45(1):38–46

Li Y, Cheng H, Duong TQ (2008) Blood-flow magnetic resonance imaging of the retina. Neuroimage 39(4):1744–1751

Li F, Liu J, Jas GS, Zhang J, Qin G, Xing J, Cotes C, Zhao H, Wang X, Diaz LA, Shi ZZ, Lee DY, Li KC, Li Z (2010) Synthesis and evaluation of a near-infrared fluorescent non-peptidic bivalent integrin alpha(v)beta(3) antagonist for cancer imaging. Bioconjug Chem 21:270–278

Li X, Xu G, Wang Y, Xu X, Liu X, Tang S, Zhang F, Zhang J, Tang L, Wu Q, Luo D, Ke X, AURORA Study Group (2014a) Safety and efficacy of conbercept in neovascular age-related macular degeneration: results from a 12-month randomized phase 2 study: AURORA study. Ophthalmology 121:1740

Li YJ, Li XH, Wang LF, Kuang X, Hang ZX, Deng Y, Du JR (2014b) Therapeutic efficacy of a novel non-peptide αvβ3 integrin antagonist for pathological retinal angiogenesis in mice. Exp Eye Res 129:119–126

Liekens S, Schols D, Hatse S (2010) CXCL12-CXCR4 axis in angiogenesis, metastasis and stem cell mobilization. Curr Pharm Des 16(35):3903–3920

Lin M, Hu Y, Chen Y, Zhou KK, Jin J, Zhu M, Le YZ, Ge J, Ma JX (2012) Impacts of hypoxia-inducible factor-1 knockout in the retinal pigment epithelium on choroidal neovascularization. Invest Ophthalmol Vis Sci 53(10):6197–6206

Linsenmeier RA, Braun RD, McRipley MA, Padnick LB, Ahmed J, Hatchell DL, McLeod DS, Lutty GA (1998) Retinal hypoxia in long-term diabetic cats. Invest Ophthalmol Vis Sci 39 (9):1647–1657

Longeras R, Farjo K, Ihnat M, Ma JX (2012) A PEDF-derived peptide inhibits retinal neovascularization and blocks mobilization of bone marrow-derived endothelial progenitor cells. Exp Diabetes Res 2012:518426

Lukason M, DuFresne E, Rubin H, Pechan P, Li Q, Kim I, Kiss S, Flaxel C, Collins M, Miller J, Hauswirth W, Maclachlan T, Wadsworth S, Scaria A (2011) Inhibition of choroidal neovascularization in a nonhuman primate model by intravitreal administration of an AAV2 vector expressing a novel anti-VEGF molecule. Mol Ther 19(2):260–265

Luna JD, Chan C-C, Derevjanik NL, Mahlow J, Chiu C, Peng B, Tobe T, Campochiaro PA, Vinores SA (1997) Blood-retinal barrier (BRB) breakdown in experimental autoimmune uveoretinitis: comparison with vascular endothelial growth factor, tumor necrosis factor, and interleukin-1ß-mediated breakdown. J Neurosci Res 49:268–280

Luttun A, Tjwa M, Carmeliet P (2002) Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. Ann N Y Acad Sci 979:80–93

Lutty GA, Hasegawa T, Baba T, Grebe R, Bhutto I, McLeod DS (2010) Development of the human choriocapillaris. Eye (Lond) 24(3):408–415

Ma AC, Fung TK, Lin RH, Chung MI, Yang D, Ekker SC, Leung AY (2011) Methionine aminopeptidase 2 is required for HSC initiation and proliferation. Blood 118(20):5448–5457

Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J (2008) Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 358 (21):2240–2248

Mahon PC, Hirota K, Semenza GL (2001) FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev 15(20):2675–2686

Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 105(2):659–669

Mann IC (1928) The development of the human eye. Cambridge University Press, Cambridge

Marchetti C, Gasparri ML, Ruscito I, Palaia I, Perniola G, Carrone A, Farooqi AA, Pecorini F, Muzii L, Panici PB (2015) Advances in anti-angiogenic agents for ovarian cancer treatment: the role of trebananib (AMG 386). Crit Rev Oncol Hematol 94(3):302–310

Martin DF, Maguire MG, Fine SL, Ying GS, Jaffe GJ, Grunwald JE, Toth C, Redford M, Ferris FL (2012) Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. Ophthalmology 119:1388–1398

Mauriz JL, Martín-Renedo J, García-Palomo A, Tuñón MJ, González-Gallego J (2010) Methionine aminopeptidases as potential targets for treatment of gastrointestinal cancers and other tumours. Curr Drug Targets 11(11):1439–1457

Maxwell PH (2005) Hypoxia-inducible factor as a physiological regulator. Exp Physiol 90 (6):791–797

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399(6733):271–275

McLeod DS, Lutty GA, Wajer SD, Flower RW (1987) Visualization of a developing vasculature. Microvasc Res 33:257–269

McLeod DS, Hasegawa T, Prow T, Merges C, Lutty G (2006) The initial fetal human retinal vasculature develops by vasculogenesis. Dev Dyn 235(12):3336–3347

McLeod DS, Baba T, Bhutto IA, Lutty GA (2012) Co-expression of endothelial and neuronal nitric oxide synthases in the developing vasculatures of the human fetal eye. Graefes Arch Clin Exp Ophthalmol 250(6):839–848

Michaelson IC (1948) The mode of development of the vascular system of the retina, with some observations on its significance for certain retinal diseases. Trans Ophthalmol Soc U K 68:137–181

Michels S, Rosenfeld PJ, Puliafito CA, Marcus EN, Venkatraman AS (2005) Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration twelve-week results of an uncontrolled open-label clinical study. Ophthalmology 112(6):1035–1047

Miki K, Miki A, Matsuoka M, Muramatsu D, Hackett SF, Campochiaro PA (2009) Effects of intraocular ranibizumab and bevacizumab in transgenic mice expressing human vascular endothelial growth factor. Ophthalmology 116:1748–1754

Mitchell P, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, Sutter F, Simader C, Burian G, Gerstner O, Weichselberger A, RESTORE Study Group (2011) The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for DME. Ophthalmology 118(4):615–625

Mole DR, Pugh CW, Ratcliffe PJ, Maxwell PH (2002) Regulation of the HIF pathway: enzymatic hydroxylation of a conserved prolyl residue in hypoxia-inducible factor alpha subunits governs capture by the pVHL E3 ubiquitin ligase complex. Adv Enzyme Regul 42:333–347

Morishita R, Aoki M, Hashiya N, Yamasaki K, Kurinami H, Shimizu S, Makino H, Takesya Y, Azuma J, Ogihara T (2004) Therapeutic angiogenesis using hepatocyte growth factor (HGF). Curr Gene Ther 4(2):199–206

Nakamura-Ishizu A, Kurihara T, Okuno Y, Ozawa Y, Kishi K, Goda N (2012) The formation of an angiogenic astrocyte template is regulated by the neuroretina in a HIF-1-dependent manner. Dev Biol 363:106–114

Nazari H, Zhang L, Zhu D et al (2015) Stem cell based therapies for age-related macular degeneration: the promises and the challenges. Prog Retin Eye Res 48:1–39

Ng EW, Shima DT, Calias P, Cunningham ET Jr, Guyer DR, Adamis AP (2006) Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov 5(2):123–132

Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, Gibson A, Sy J, Rundle AC, Hopkins JJ, Rubio RG, Ehrlich JS, RISE and RIDE Research Group (2012) Ranibizumab for DME: results from 2 phase III randomized trials: RISE and RIDE. Ophthalmology 119 (4):789–801

Okubo Y, Sekiya H, Namiki S, Sakamoto H, Iinuma S, Yamasaki M, Watanabe M, Hirose K, Iino M (2010) Imaging extrasynaptic glutamate dynamics in the brain. Proc Natl Acad Sci U S A 107:6526–6531

Ozaki H, Seo MS, Ozaki K, Yamada H, Yamada E, Okamoto N, Hofmann F, Wood JM, Campochiaro PA (2000) Blockage of vascular endothelial cell growth factor receptor signaling is sufficient to completely prevent retinal neovascularization. Am J Pathol 156:697–707

Papadopoulos N, Martin J, Ruan Q, Rafique A, Rosconi MP, Shi E, Pyles EA, Yancopoulos GD, Stahl N, Wiegand SJ (2012) Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. Angiogenesis 15:171–185

Park H, Lee DS, Yim MJ, Choi YH, Park S, Seo SK, Choi JS, Jang WH, Yea SS, Park WS, Lee CM, Jung WK, Choi IW (2015) 3,3'-Diindolylmethane inhibits VEGF expression through the HIF-1α and NF-kB pathways in human retinal pigment epithelial cells under chemical hypoxic conditions. Int J Mol Med 36(1):301–308

Pechan P, Rubin H, Lukason M, Ardinger J, DuFresne E, Hauswirth WW, Wadsworth SC, Scaria A (2009) Novel anti-VEGF chimeric molecules delivered by AAV vectors for inhibition of retinal neovascularization. Gene Ther 16(1):10–16

Peiris PM, Toy R, Doolittle E, Pansky J, Abramowski A, Tam M, Vicente P, Tran E, Hayden E, Camann A, Mayer A, Erokwu BO, Berman Z, Wilson D, Baskaran H, Flask CA, Keri RA, Karathanasis E (2012) Imaging metastasis using an integrin-targeting chain-shaped nanoparticle. ACS Nano 6:8783–8795

Penfold PL, Provis JM, Madigan MC, van Driel D, Billson FA (1990) Angiogenesis in normal human retinal development: the involvement of astrocytes and macrophages. Graefes Arch Clin Exp Ophthalmol 228(3):255–263

Pershing S, Bakri SJ, Moshfeghi DM (2013) Ocular hypertension and intraocular pressure asymmetry after intravitreal injection of anti-vascular endothelial growth factor agents. Ophthalmic Surg Lasers Imaging Retina 44:460–464

Phelps DL (1990) Oxygen and developmental retinal capillary remodeling in the kitten. Invest Ophthalmol Vis Sci 31:2194–2200

Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M (2005) Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev 16:159–178

Provis JM, Leech J, Diaz CM, Penfold PL, Stone J, Keshet E (1997) Development of the human retinal vasculature: cellular relations and VEGF expression. Exp Eye Res 65:555–568

Querques G, Capuano V, Frascio P, Bandello F, Souied EH (2015) Emerging therapeutic options in age-related macular degeneration. Ophthalmic Res 53(4):194–199

Rajendram R, Fraser-Bell S, Kaines A, Michaelides M, Hamilton RD, Esposti SD, Peto T, Egan C, Bunce C, Leslie RD, Hykin PG (2012) A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. Arch Ophthalmol 130(8):972–979

Rakoczy EP, Lai CM, Magno AL, Wikstrom ME, French MA, Pierce CM, Schwartz SD, Blumenkranz MS, Chalberg TW, Degli-Esposti MA, Constable IJ (2015) Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial. Lancet 386(10011):2395–2403

Ratcliffe PJ (2007) HIF-1 and HIF-2: working alone or together in hypoxia? J Clin Invest 117 (4):862–865

Reck M, Kaiser R, Mellemgaard A, Douillard JY, Orlov S, Krzakowski M, von Pawel J, Gottfried M, Bondarenko I, Liao M, Gann CN, Barrueco J, Gaschler-Markefski B, Novello S, LUME-Lung 1 Study Group (2014) Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. Lancet Oncol 15:143–155

Rich RM, Rosenfeld PJ, Puliafito CA, Dubovy SR, Davis JL, Flynn HW Jr, Gonzalez S, Feuer WJ, Lin RC, Lalwani GA, Nguyen JK, Kumar G (2006) Short-term safety and efficacy of intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Retina 26(5):495–511

Rizzolo LJ (1997) Polarity and the development of the outer blood-retinal barrier. Histol Histopathol 12:1057–1067

Rodrigues M, Xin X, Jee K, Babapoor-Farrokhran S, Kashiwabuchi F, Ma T, Bhutto I, Hassan SJ, Daoud Y, Baranano D, Solomon S, Lutty G, Semenza GL, Montaner S, Sodhi A (2013) VEGF secreted by hypoxic Müller cells induces MMP-2 expression and activity in endothelial cells to promote retinal neovascularization in proliferative diabetic retinopathy. Diabetes 62 (11):3863–3873

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY, MARINA Study Group (2006) Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 355(14):1419–1431

Roy-Luzarraga M, Hodivala-Dilke K (2016) Molecular pathways: endothelial cell FAK-A target for cancer treatment. Clin Cancer Res 22(15):3718–3724

Rubio R (2014) Long-acting anti-VEGF delivery. Retina Today: 78–80

Ruzinova MB, Schoer RA, Gerald W, Egan JE, Pandolfi PP, Rafii S, Manova K, Mittal V, Benezra R (2003) Effect of angiogenesis inhibition by Id loss and the contribution of bone-marrow-derived endothelial cells in spontaneous murine tumors. Cancer Cell 4(4):277–289

Sacu S, Michels S, Prager F, Weigert G, Dunavoelgyi R, Geitzenauer W, Pruente C, Schmidt-Erfurth U (2009) Randomised clinical trial of intravitreal Avastin vs photodynamic therapy and intravitreal triamcinolone: long-term results. Eye (Lond) 23(12):2223–2227

Salehi-Had H, Roh MI, Giani A, Hisatomi T, Nakao S, Kim IK, Gragoudas ES, Vavvas D, Guccione S, Miller JW (2011) Utilizing targeted gene therapy with nanoparticles binding alpha v beta 3 for imaging and treating choroidal neovascularization. PLoS One 6(4), e18864

Sampieri CL, León-Córdoba K, Remes-Troche JM (2013) Matrix metalloproteinases and their tissue inhibitors in gastric cancer as molecular markers. J Cancer Res Ther 9(3):356–363

Sandercoe TM, Madigan MC, Billson FA, Penfold PL, Provis JM (1999) Astrocyte proliferation during development of the human retinal vasculature. Exp Eye Res 69:511–523

Sarlos S, Rizkalla B, Moravski CJ, Cao Z, Cooper ME, Wilkinson-Berka JL (2003) Retinal angiogenesis is mediated by an interaction between the angiotensin type 2 receptor, VEGF, and angiopoietin. Am J Pathol 163(3):879–887

Scarpino S, D'Alena FC, Di Napoli A, Ballarini F, Prat M, Ruco LP (2003) Papillary carcinoma of the thyroid: evidence for a role for hepatocyte growth factor (HGF) in promoting tumour angiogenesis. J Pathol 199(2):243–250

Schofield CJ, Ratcliffe PJ (2004) Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5 (5):343–354

Scholz CC, Taylor CT (2013) Targeting the HIF pathway in inflammation and immunity. Curr Opin Pharmacol 13(4):646–653

Schor AM, Schor SL (2010) Angiogenesis and tumour progression: migration-stimulating factor as a novel target for clinical intervention. Eye (Lond) 24(3):450–458

Semenza GL (2007) Vasculogenesis, angiogenesis, and arteriogenesis: mechanisms of blood vessel formation and remodeling. J Cell Biochem 102(4):840–847

Semenza GL (2012) Hypoxia-inducible factors in physiology and medicine. Cell 148(3):399–408

Sen D, SoRelle ED, Liba O, Dalal R, Paulus YM, Kim T-W, Moshfeghi DM, de la Zerda A (2016) High resolution contrast-enhanced optical coherence tomography in mice retinae. J Biomed Opt 21(6):066002

Sheetz MJ, Aiello LP, Davis MD, Danis R, Bek T, Cunha-Vaz J, Shahri N, Berg PH, MBDL and MBCU Study Groups (2013) The effect of the oral PKC β inhibitor ruboxistaurin on vision loss in two phase 3 studies. Invest Ophthalmol Vis Sci 54(3):1750–1757

Shih SC, Ju M, Liu N, Smith LE (2003) Selective stimulation of VEGFR-1 prevents oxygen-induced retinal vascular degeneration in retinopathy of prematurity. J Clin Invest 112(1):50–57

Simard JR, Getlik M, Grütter C, Pawar V, Wulfert S, Rabiller M, Rauh D (2009) Development of a fluorescent-tagged kinase assay system for the detection and characterization of allosteric kinase inhibitors. J Am Chem Soc 131:13286–13296

Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, D'Amore PA (1994) Oxygen-induced retinopathy in the mouse. Invest Ophthalmol Vis Sci 35(1):101–111

Sodhi A, Montaner S (2015) Angiopoietin-like 4 as an emerging therapeutic target for diabetic Eye disease. JAMA Ophthalmol 133(12):1375–1376

Solomon SD, Lindsley K, Vedula SS, Krzystolik MG, Hawkins BS (2014) Anti-vascular endothelial growth factor for neovascular age-related macular degeneration. Cochrane Database Syst Rev 8, CD005139

Stalmans I, Ng YS, Rohan R, Fruttiger M, Bouché A, Yuce A, Fujisawa H, Hermans B, Shani M, Jansen S, Hicklin D, Anderson DJ, Gardiner T, Hammes HP, Moons L, Dewerchin M, Collen D, Carmeliet P, D'Amore PA (2002) Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. J Clin Invest 109:327–336

Stefánsson E, Machemer R, de Juan E Jr, McCuen BW II, Peterson J (1992) Retinal oxygenation and laser treatment in patients with diabetic retinopathy. Am J Ophthalmol 113(1):36–38

Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, Keshet E (1995) Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression in neuroglia. J Neurosci 15:4738–4747

Subhani S, Vavilala DT, Mukherji M (2016) HIF inhibitors for ischemic retinopathies and cancers: options beyond anti-VEGF therapies. Angiogenesis 19(3):257–273

Subramanian ML, Abedi G, Ness S, Ahmed E, Fenberg M, Daly MK, Houranieh A, Feinberg EB (2010) Bevacizumab vs ranibizumab for age-related macular degeneration: 1-year outcomes of a prospective, double-masked randomised clinical trial. Eye (Lond) 24(11):1708–1715

Sun D, Nakao S, Xie F, Zandi S, Bagheri A, Kanavi MR, Samiei S, Soheili ZS, Frimmel S, Zhang Z, Ablonczy Z, Ahmadieh H, Hafezi-Moghadam A (2014) Molecular imaging reveals elevated VEGFR-2 expression in retinal capillaries in diabetes: a novel biomarker for early diagnosis. FASEB J 28(9):3942–3951

Takeda A, Baffi JZ, Kleinman ME, Cho WG, Nozaki M, Yamada K, Kaneko H, Albuquerque RJ, Dridi S, Saito K, Raisler BJ, Budd SJ, Geisen P, Munitz A, Ambati BK, Green MG, Ishibashi T, Wright JD, Humbles AA, Gerard CJ, Ogura Y, Pan Y, Smith JR, Grisanti S, Hartnett ME, Rothenberg ME, Ambati J (2009) CCR3 is a target for age-related macular degeneration diagnosis and therapy. Nature 460:225–230

Thakur A, Scheinman RI, Rao VR, Kompella UB (2011) Pazopanib, a multitargeted tyrosine kinase inhibitor, reduces diabetic retinal vascular leukostasis and leakage. Microvasc Res 82 (3):346–350

The Branch Vein Occlusion Study Group (1984) Argon laser photocoagulation for macular edema in branch vein occlusion. Am J Ophthalmol 98(3):271–282

The Diabetic Retinopathy Study Research Group (1981) Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings. DRS report number 8. Ophthalmology 88(7):583–600

Todorich B, Yiu G, Hahn P (2014) Current and investigational pharmacotherapeutic approaches for modulating retinal angiogenesis. Expert Rev Clin Pharmacol 7(3):375–391

Tolentino MJ (2009) Current molecular understanding and future treatment strategies for pathologic ocular neovascularization. Curr Mol Med 9(8):973–981

Treps L, Conradi LC, Harjes U, Carmeliet P (2016) Manipulating angiogenesis by targeting endothelial metabolism: hitting the engine rather than the drivers – a new perspective? Pharmacol Rev 68(3):872–887

Tripathi BJ, Tripathi RC (1997) Development of the human eye. In: Bron AJ, Tripathi RC, Tripathi BJ (eds) Wolff's anatomy of the eye and orbit, 8th edn. Chapman & Hall, London

Tufail A, Patel PJ, Egan C, Hykin P, da Cruz L, Gregor Z, Dowler J, Majid MA, Bailey C, Mohamed Q, Johnston R, Bunce C, Xing W, ABC Trial Investigators (2010) Bevacizumab for neovascular age related macular degeneration (ABC Trial): multicentre randomised double masked study. BMJ 340:c2459

Tzeng HE, Chen PC, Lin KW, Lin CY, Tsai CH, Han SM, Teng CL, Hwang WL, Wang SW, Tang CH (2015) Basic fibroblast growth factor induces VEGF expression in chondrosarcoma cells and subsequently promotes endothelial progenitor cell-primed angiogenesis. Clin Sci (Lond) 129(2):147–158

Varner JA, Cheresh DA (1996) Tumor angiogenesis and the role of vascular cell integrin alphavbeta3. Important Adv Oncol 69–87

Vinores SA (1995) Assessment of blood-retinal barrier integrity. Histol Histopathol 10:141–154

Vinores SA, Derevjanik NL, Ozaki H, Okamoto N, Campochiaro PA (1999) Cellular mechanisms of blood-retinal barrier dysfunction in macular edema. Doc Ophthalmol 97(3-4):217–228

Vrabec JP, Lieven CJ, Levin LA (2003) Cell-type-specific opening of the retinal ganglion cell mitochondrial permeability transition pore. Invest Ophthalmol Vis Sci 44:2774–2782

Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 92 (12):5510–5514

Wang Q, Li T, Wu Z, Wu Q, Ke X, Luo D, Wang H (2013) Novel VEGF decoy receptor fusion protein conbercept targeting multiple VEGF isoforms provide remarkable anti-angiogenesis effect in vivo. PLoS One 8, e70544

Wilkinson-Berka JL, Rana I, Armani R, Agrotis A (2013) Reactive oxygen species, Nox and angiotensin II in angiogenesis: implications for retinopathy. Clin Sci (Lond) 124(10):597–615

Wu P, Nielsen TE, Clausen MH (2015) FDA-approved small-molecule kinase inhibitors. Trends Pharmacol Sci 36(7):422–439

Xie F, Luo W, Zhang Z, Sun D (2012) In vivo molecular imaging in retinal disease. J Ophthalmol 2012:429387

Xin X, Rodrigues M, Umapathi M, Kashiwabuchi F, Ma T, Babapoor-Farrokhran S, Wang S, Hu J, Bhutto I, Welsbie DS, Duh EJ, Handa JT, Eberhart CG, Lutty G, Semenza GL, Montaner S, Sodhi A (2013) Hypoxic retinal Muller cells promote vascular permeability by HIF-1-dependent up-regulation of angiopoietin-like 4. Proc Natl Acad Sci U S A 110(36):E3425–E3434

Yan F, Wu H, Liu H, Deng Z, Liu H, Duan W, Liu X, Zheng H (2016) Molecular imaging-guided photothermal/photodynamic therapy against tumor by iRGD-modified indocyanine green nanoparticles. J Control Release 224:217–228

Yao Y, Tsirka SE (2011) Truncation of monocyte chemoattractant protein 1 by plasmin promotes blood-brain barrier disruption. J Cell Sci 124(Pt 9):1486–1495

Ye X, Wang Y, Nathans J (2010) The Norrin/Frizzled4 signaling pathway in retinal vascular development and disease. Trends Mol Med 16(9):417–425

Yu DY, Cringle SJ (2001) Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. Prog Retin Eye Res 20:175–208

Zhang X, Huang S, Guo J, Zhou L, You L, Zhang T, Zhao Y (2016) Insights into the distinct roles of MMP-11 in tumor biology and future therapeutics (Review). Int J Oncol 48(5):1783–1793
Zhou AY, Bai YJ, Zhao M, Yu WZ, Huang LZ, Li XX (2014) Placental growth factor expression is reversed by antivascular endothelial growth factor therapy under hypoxic conditions. World J Pediatr 10(3):262–270

Corticosteroids and Anti-Complement Therapy in Retinal Diseases

Raja Narayanan and Baruch D. Kuppermann

Contents

Abstract

Corticosteroids are unique in that they are the one class of agents that acts upon most of the multiple processes in the pathophysiology of macular edema. Corticosteroids are capable of inhibiting prostaglandin and leukotriene synthesis as well as interfering with intercellular adhesion molecule-1 (ICAM-1), interleukin-6, VEGF-A, and stromal cell derived factor-1 pathways. Triamcinolone, dexamethasone, and fluocinolone have been extensively used in the treatment of retinal and choroidal vascular diseases. Sustained release implants of steroids have reduced the burden of repeated intravitreal injections necessary in most of the retinal diseases. Complement factors play an important role in the pathogenesis of age-related macular degeneration (AMD). Inhibitors of complement could provide a breakthrough in the treatment of dry AMD. Complement factor inhibitors, such as POT-4, lampalizumab, and eculizumab, have been tested in clinical trials for dry AMD with promising results. However, results of phase 3 trials are awaited.

R. Narayanan
Smt Kanuri Santhamma Vitreoretina Center, L.V. Prasad Eye Institute, Hyderabad, India

B.D. Kuppermann (✉)
UC Irvine Medical Center, Ophthalmology Clinic, Orange, CA, USA
e-mail: bdkupper@uci.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_22

Keywords
Complement • Dexamethasone • Eculizumab • Fluocinolone • Lampalizumab • Steroids • Triamcinolone

1 Corticosteroids

This chapter provides an extensive description of major clinical trials of intravitreal steroids in diabetic macular edema (DME), retinal venous occlusion (RVO), and dry age-related macular degeneration (AMD)

Chronic, low-grade inflammation appears to be a significant factor in the causation of macular edema due to DME, RVO, and AMD. The goals of therapy for macular edema should be to reduce inflammation, restore blood–retinal barrier patency, and interfere with the production or action of vascular endothelial growth factor (VEGF) and other proinflammatory cytokines. Corticosteroids are unique in that they are the one class of agents that acts upon most of the multiple processes in the pathophysiology of macular edema. For example, corticosteroids are capable of inhibiting prostaglandin and leukotriene synthesis as well as interfering with intercellular adhesion molecule-1 (ICAM-1), interleukin-6, VEGF-A, and stromal cell derived factor-1 pathways (Tamura et al. 2005). Corticosteroids have also been shown to decrease paracellular permeability and increase tight junction integrity both by directly restoring tight junctional proteins to their proper location at the cell border and by increasing the gene expression of those proteins (Felinski and Antonetti 2005; Antonetti et al. 2002). Steroids have been extensively used to treat macular edema (ME) due to diabetic retinopathy (DR) and RVO.

The mechanisms by which high glucose levels directly lead to diabetic retinopathy are not well known. Activation of protein kinase C, accumulation of polyols through the aldose reductase pathway, increased formation of advanced glycation end products, and overproduction of free radicals may occur due to hyperglycemia (Tang and Kern 2011). These metabolic changes increase proinflammatory cytokines, chemokines, and other inflammatory mediators that stimulate an influx of leukocytes and alter vascular permeability (Tang and Kern 2011). Elevated levels of interleukin 6 (IL-6), IL-8, tumor necrosis factor-α (TNFα), VEGF, interferon-induced protein-10 (IP-10), intercellular adhesion molecule 1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1) have been demonstrated in eyes with DR (Tang and Kern 2011). A number of inflammatory cytokines and growth factors may be elevated in RVO patients, including IL-1α, IL-6, IL-8, MCP-1, platelet derived growth factor (PDGF-) AA, and VEGF relative to control eyes (Lee et al. 2012; Noma et al. 2005).

Genetic studies have implicated the complement system as well as other immune responses in disease pathogenesis and severity. Histologic studies have shown the presence of macrophages, lymphocytes, and mast cells, as well as fibroblasts in dry AMD (Whitcup et al. 2013a). With aging, oxidative stress secondary to the accumulation of oxidized lipoproteins and free radicals in retinal and choroidal tissues

Table 1 Relative potency of Steroids

Steroid	Relative potency
Hydrocortisone	1.0
Prednisolone	4.0
Methylprednisolone	5
Triamcinolone	5
Dexamethasone	26
Betamethasone	33

may trigger a tissue adaptive response, recently described as *para*-inflammation (Xu et al. 2009). Sustained injury or chronic inflammation may lead to an imbalance in the local inflammatory response and contribute to AMD (Whitcup et al. 2013b; Ozaki et al. 2014). Reactive oxygen species induced injury to RPE cells may release cytokines and chemokines that recruit and activate choroidal dendritic cells. This may amplify the inflammatory process leading to additional RPE cell damage, potentially producing a state of chronic inflammation (Hageman et al. 2001). Knockout of antioxidative genes in mice (Sod1−/− mice, Sod2 knockdown mice, and Nrf2−/− mice) results in the development of the typical features of AMD (Hashizume et al. 2008; Zhao et al. 2011). Genetically, polymorphism in a number of genes, including members of the complement pathway, apolipoprotein E (ApoE), ARMS2, HTRA1, CX3CR1, VEGF-A, and ABCA4 have been associated with AMD, indicating the involvement of inflammation, lipid metabolism, RPE dysfunction, and angiogenesis in AMD (Ding et al. 2009).

The various intraocular steroids that have been used include dexamethasone, triamcinolone, and fluocinolone (Table 1)

1.1 Diabetic Macular Edema

1.1.1 Intravitreal Triamcinolone

Intravitreal triamcinolone (IVTA) has been used in many small case series and randomized clinical trials (RCTs). These studies were later evaluated in a Cochrane review, the data of which showed some favorable results, although IVTA is associated with an increased risk of glaucoma and cataract, as is the case with all the available steroid products for retinal use to 1° or another.

The DRCR.net compared the efficacy and safety of preservative-free IVTA to focal/grid laser photocoagulation for the treatment of DME in a follow-up period of 2 years, which was then extended to 3 years (Diabetic Retinopathy Clinical Research Network 2008). Patients were randomized to three arms to receive focal/grid laser photocoagulation, IVTA 1 mg, or IVTA 4 mg, respectively. Even though greater gain in BCVA was reported with IVTA 4-mg at 4 months, no significant differences in BCVA were noticed among the three groups after the first year. However, at the 2-year endpoint, mean BCVA was better in the laser

group, and this result was also confirmed by the central retinal thickness (CRT) measurements from optical coherence tomography.

In the DRCR protocol I study, patients were randomized to four arms: sham injection plus laser, 0.5 mg of intravitreal ranibizumab (IVR) plus prompt laser, 0.5 mg IVR plus deferred laser, and 4 mg IVTA plus prompt laser. At the first year endpoint data showed a greater benefit in terms of BCVA gain in the groups with IVR plus prompt or deferred laser compared to the arms with IVTA plus laser and laser alone; the three study groups evaluating IVTA or IVR plus laser also revealed similar results in CRT measurements, with a greater reduction in CRT compared to the group with laser alone (Diabetic Retinopathy Clinical Research Network et al. 2010). However, in the pseudophakic subgroup analysis, the results of IVTA were equivalent to IVR groups with far fewer injections. At the extended 2-year follow-up, the results were consistent with those published after the first year (Elman et al. 2011). Compared to the group with laser alone, the mean change in BCVA was +3.7 letters better in the group with IVR plus prompt laser, +5.8 letters greater in the group with IVR plus deferred laser, and −1.5 letters worse in the group with IVTA plus prompt laser.

1.1.2 Dexamethasone Implant

Dexamethasone intravitreal implant (DEX implant, Ozurdex®; Allergan) is a biodegradable device containing 0.7 mg of preservative-free dexamethasone. The device is injected into the vitreous through a prefilled, single-use, 22-gauge applicator, and it was engineered to deliver the drug with slow-release kinetics.

The efficacy and safety of the DEX implant has been evaluated in patients with DME. Based on the MEAD study, sustained-release of 0.7 mg dexamethasone intravitreal implant (Ozurdex; Allergan, Irvine, CA, USA) received US FDA approval for treating DME (Boyer et al. 2014). The MEAD study randomized 1,048 DME patients to study treatment with Ozurdex 0.7 mg, 0.35 mg, or sham procedure. At 3 years, 22.2%, 18.4%, and 12% of eyes improved by 15 letters or more in BCVA from baseline in the 0.7 mg, 0.35 mg, and sham group, respectively. The most common ocular adverse events were cataract and increased IOP. Overall incidence of cataract-related adverse events was 67.9%, 64.1%, and 20.4% in the 0.7 mg, 0.35 mg, and sham group, respectively. A steroid-induced IOP increase was seen in approximately one-third of patients, and 41.5% and 37.6% required IOP lowering medication in the 0.7 mg and 0.35 mg groups, respectively.

Maturi et al. conducted a 12-month, randomized, controlled study to evaluate the effects of a 0.7 mg DEX implant combined with bevacizumab compared to bevacizumab monotherapy in the DME patients, who had previously responded poorly to bevacizumab (2015). The average number of bevacizumab injections was six in the combination group and nine in the bevacizumab only group. However, the combination group needed an average number of 2.1 DEX implant treatments. The first implant was injected at month 1, and additional implants were injected at months 5 and 9. At the end of 1 year, an increase in the BCVA was reported in the two groups, but did not significantly differ between the groups. The combination group had a significantly reduced CRT from the monotherapy group (Fig. 1).

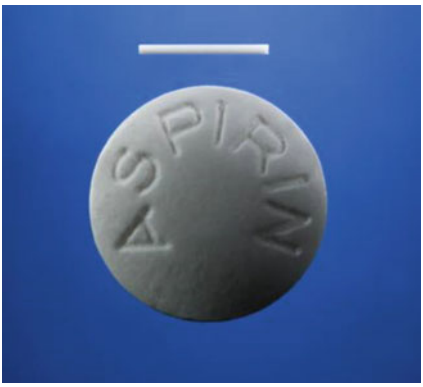

Fig. 1 Comparative size of OZURDEX

The BEVORDEX study was the first head-to-head comparison of DEX versus intravitreal bevacizumab (1.25 mg) for DME patients (88 eyes, 61 patients) (Gillies et al. 2014). The primary outcome was the proportion of eyes that improved in BCVA by 10 logMAR letters, which was achieved in 40% in the bevacizumab group versus 41% of the dexamethasone-treated eyes. At 1 year, superior anatomic outcomes were found in the DEX group (mean CMT reduction was 122.1 microns in the bevacizumab group vs. 187.1 microns in the DEX group). However, 11% (five of 46) of DEX-treated eyes lost vision (decrease of 10 letters or more), mainly because of cataract, versus none of the 42 eyes treated with bevacizumab. The bevacizumab group received a mean of 8.6 injections compared with 2.7 in the DEX group.

Zhioua et al. reported favorable outcomes after DEX implant in cases of ranibizumab resistant DME (2015). Resistant DME was defined as poor response after 6 monthly injections of ranibizumab. The mean visual acuity improved by approximately 5 letters at 6 months, and the central macular thickness reduced by approximately 30%. Similar results have been shown in many other real-life studies.

1.1.3 DEX in Vitrectomized Eyes

The pharmacokinetic profile of the 0.7 mg DEX implant in vitrectomized and nonvitrectomized eyes was studied in an animal model by Chang-Lin et al. (2011). In this study, the concentration of DEX in the vitreous fluid and retina did not differ statistically significantly between the vitrectomized and nonvitrectomized eyes during each visit of 31 days of follow-up. The OZURDEX CHAMPLAIN study assessed the efficacy and safety of the 0.7 mg DEX implant in vitrectomized patients with treatment-resistant DME (Boyer et al. 2011). In this prospective, multicenter, 26-week, Phase II trial, the patients ($n = 55$) had a mean duration of DME for 43 months and had pars plana vitrectomy 31 months prior to the study. The maximum effect for the decrease of the mean CRT was obtained at week 8, and it was statistically significantly lower than the baseline during the study. An increase in the BCVA from the baseline was observed at week 1, and the

Fig. 2 Iluvien implant

mean gain in the letters was found to be +6.0 at week 8 and +3.0 letters at week 26. At the end of the study, 43% of the patients gained at least 5 letters and 21% had gained at least 10 letters. A loss of $10 letters was observed in 11% and loss of $15 letters in 7% the patients at the final visit.

1.1.4 Fluocinolone Acetonide Implant

Iluvien is a small, long-lasting non-biodegradable insert containing 250 μg of fluocinolone acetonide. It is injected into the vitreous through a 25-gauge needle and engineered to release 0.5 or 0.2 μg/day of the active principle. The efficacy of Iluvien insert for the treatment of DME resistant to laser was evaluated in recent RCTs, with a 3-year follow-up period (FAME studies) (Fig. 2) (Campochiaro et al. 2012).

Patients with persistent DME and at least one prior laser treatment were randomly assigned to three arms: 0.2 μg/day (low dose), 0.5 μg/day (high dose), or sham injection. Data showed a BCVA improvement of 15 or more letters in 28.7%, 27.8%, and 18.9% of the three groups, respectively, at both 24 and 36 months. Retreatment was possible after 12 months and it was performed in 25% of patients (with a mean number of 4 inserts over 3 years).

Moreover, about 40% of patients underwent rescue laser treatment. In addition, to evaluate whether the duration of DME could play a role in determining the response to therapy, a further analysis has been developed which clearly revealed that the response to fluocinolone acetonide was different in patients with longer or shorter duration DME. In particular, for patients with a mean disease duration of 3 or more years, the number of significant responses (BCVA gain of 15 or more letters) was significantly higher in the group treated with the steroid implant compared to the sham control group. In contrast, the number of patients with a mean duration of DME of less than 3 years who presented an adequate BCVA improvement (15 or more letters) was not significantly different from the control group. However both shorter and longer lasting DME patients had good responses to the fluocinolone implant; rather the variability was in the sham control group. Unfortunately, side effects were frequent; almost all phakic eyes required cataract extraction and the rate of glaucoma needing surgical intervention was 4.8%, and the overall rate of IOP lowering medication use was approximately 40%. The

fluocinolone acetonide is approved by the FDA for the treatment of DME in patients who were previously treated with a course of corticosteroids and exhibited no clinically significant rise in IOP.

1.2 Retinal Vein Occlusion

The SCORE study (The Standard Care versus Corticosteroid for REtinal vein occlusion) consists of two multicenter, randomized, Phase III trials. The SCORE-CRVO study compared two different doses of IVTA (1 and 4 mg) to the standard of care, represented by observation, in 271 eyes with CRVO (Ip et al. 2009). At 12 months, the proportion of patients who experienced a visual acuity gain of 15 letters or more was similar among the three groups (27% in the group treated with triamcinolone 1 mg, 26% in the group treated with the 4 mg dose, and 7% in the control group). At month 24, however, a loss in visual acuity letter scores of 15 letters or more was noted in 48% of the observation group versus 31% of the triamcinolone 1 mg group and 26% of the triamcinolone group 4 mg group.

The SCORE-BRVO study enrolled 411 patients with macular edema secondary to BRVO and evaluated the efficacy of two different doses of IVTA (1 and 4 mg) compared with grid laser photocoagulation (Scott et al. 2009). There was no significant difference in visual acuity among the three groups at 12 months. The percentage of eyes gaining >3 Early Treatment Diabetic Retinopathy Study lines was 26%, 27%, and 29%, respectively. All three study groups showed OCT-measured center point thickness decreases from baseline throughout follow-up. GENEVA study (Global Evaluation of Implantable Dexamethasone in RVO with macular oedema) included two identical, multicenter, prospective, randomized, double-blind, 6-month, and sham-controlled clinical trials. A total of 1,267 patients with vision loss due to macular edema associated with BRVO or CRVO were enrolled. In the 6-month initial treatment phase, patients were randomized to the administration of dexamethasone 700 μg ($n = 427$) or dexamethasone 350 μg ($n = 414$) or sham ($n = 426$) (Haller et al. 2010). The proportion of patients that achieved an improvement in visual acuity of 15 or more letters was 22% in the 700 μg group, 23% in the 350 μg group, and 13% in the sham group at month 3. These data were no longer statistically significant at month 6. Eyes receiving dexamethasone implant 0.7 or 0.35 mg achieved a 15-letter improvement in BCVA significantly faster than the eyes receiving sham treatment. The cumulative response rate for the time to reach a 15-letter improvement from baseline BCVA was 41% in the dexamethasone implant 0.7 mg group, 40% in the 0.35 mg group, and 23% in the sham group. The reduction in mean CRT was 208 ± 201 μm in the 700 μg group, 177 ± 197 μm in the 350 μg group, and 85 ± 173 μm in the sham group at month 3 at day 180, but not statistically significant at month 6.

1.3 Age-Related Macular Degeneration

The LuceDex prospective randomized pilot trial compared the combination of IVR and dexamethasone with ranibizumab monotherapy for the treatment of neovascular age-related macular degeneration (nvAMD) (Ranchod et al. 2013; Kuppermann et al. 2015). Subjects were randomized 1:1 to combination therapy or monotherapy. Group 1 (combination therapy) received treatments comprised of intravitreal dexamethasone 500 mg in 0.05 mL followed by IVR 0.5 mg in 0.05 mL. Group 2 (monotherapy) received only IVR 0.5 mg in 0.05 mL. Study eyes in both groups received the study treatment monthly for 4 months followed by treatment on indication. A visual acuity gain of at least zero letters from baseline to Month 12 was achieved by 15 patients (88%) in Group 1 and 14 patients (70%) in Group 2. A gain of at least 15 letters from baseline to Month 12 was achieved by 6 patients (35%) in Group 1 and 4 patients (20%) in Group 2. Mean visual acuity gain from baseline to Month 12 was 11.1 letters in Group 1 and 5.9 letters in Group 2. The mean number of treatments required at 12 months was 7.1 in Group 1 and 6.6 in Group 2 ($P = 0.23$).

The ERIE study evaluated the efficacy and safety of dexamethasone intravitreal implant 0.7 mg (DEX) as adjunctive therapy to ranibizumab in nvAMD. This was a 6-month, single masked, and multicenter study. Patients were randomized to DEX implant ($n = 123$) or sham procedure ($n = 120$) and received 2 protocol-mandated IVR injections. The main outcome measure was injection-free interval to first as-needed ranibizumab injection. DEX increased the injection-free interval versus sham (50th percentile, 34 vs. 29 days; 75th percentile, 85 vs. 56 days). 8.3% of DEX versus 2.5% of sham-treated patients did not require rescue ranibizumab ($p = 0.048$). Visual acuity and retinal thickness outcomes were similar in DEX and sham-treated patients.

1.4 Complement Inhibitors for ARMD

The complement system is considered part of the humoral component of the immunity-related nonspecific inflammatory cascade. It works by inducing inflammation, opsonizing foreign pathogens, destroying foreign cells, and removing neutralized foreign pathogens. There are three main complement pathways. In the classic complement pathway, antibody-coated targets and antigen-antibody complexes cause the Fc receptor of antigen-activated antibody molecules to bind and activate C1. In the alternative pathway, microbial surface constituents such as polysaccharides activate C3 convertase, which causes proteolysis of C3. In the mannose-binding lectin pathway, lectin can bind to mannose residues on pathogens such as viruses and also activate C3 convertase.

The only treatment for GA that has shown positive results in clinical trials at this point in time has been the anti-inflammatory treatment lampalizumab (Genentech/Roche, South San Francisco, CA, USA). However, other anti-inflammatory treatments have been trialed or are currently under investigation. Fluocinolone

(Iluvien; Alimera Sciences, Alpharetta, GA, USA) is a steroid. Glatiramer acetate (Copaxone; Teva Pharmaceuticals, Kfar-Saba, Israel) is an anti-inflammatory drug aimed at decreasing amyloid-related inflammation. Sirolimus (Rapamycin; Wyeth, Madison, WI, USA) is an antifungal which has yet to show positive results, though testing is ongoing. Another complement inhibitor which inhibits the action of C5, LFG316 (Novartis Pharmaceutical Corporation, East Hanover, NJ, USA) is currently under study. POT-4 (Appellis Pharmaceuticals, Crestwood, KY, USA and Alcon Research Ltd) acts by inhibiting the formation of C3a and C3b from C3, inhibiting the classic, alternative, and mannose-binding lectin pathways of the complement system. Eculizumab (SOLIRIS, Alexion Pharmaceuticals, Cheshire, CT, USA) is an inhibitor of C5 that prevents the formation of the membrane attack complexes at the bottom of the complement cascade. Recent investigation has not met its primary endpoint. ARC-1905 (Ophthotech, Princeton, NJ, USA) also targets C5, and has been studied in AMD patients.

Factor D is a critical early component of the alternative pathway that involves CFH. Factor D serves as the rate-limiting step of the alternative pathway, and it is present in lower plasma concentrations than other complement factors. Factor D is responsible for cleaving its substrate, factor B, prior to its association with C3. After factor D-mediated cleavage, factor B converts into the proteolytically active factor Bb that initiates the alternative pathway and activates important convertases. Factor D is upstream of factor B and other critical AMD-associated proteins, including C3, CFH, and CFI. Recent analyses of single nucleotide polymorphisms within the factor D have shown increased factor D levels in AMD patients compared with controls.

Lampalizumab (Genentech/Roche) is an antigen-binding fragment derived from a humanized monoclonal antibody to factor D. A Phase III trial of lampalizumab, a factor D inhibitor, is currently underway. Factor D was selected as a target given its location in the complement cascade, and it is present in lower abundance than C3.

MAHALO tested lampalizumab dosed monthly or bimonthly against sham injections. Patients were divided into two sham injection groups (monthly and bimonthly, both with $N = 21$), and two treatment groups receiving lampalizumab 10 mg monthly ($N = 43$) and bimonthly ($N = 44$). Treatments in all four subgroups were concluded after 18 months in all 129 patients. The primary endpoint for the Phase II trial was mean change in geographic area from baseline to month 18 on fundus autofluorescence (http://www.roche.com/investors/updates/inv-update-2013-08-27.htm). The authors also looked at mean change from baseline in GA area within three subgroups of patients: those who had less than 10 mm^2 of GA, those who started with more than 10 mm^2 of GA, and those with genetic markers determined prior to randomization. Inclusion criteria for MAHALO included bilateral GA secondary to AMD in the absence of neovascularization. BCVA by the ETDRS testing was between 20/50 and 20/400 (Snellen equivalent). GA had to be between one and seven disc areas (2.5–17.5 mm^2). If GA was multifocal, then at least one lesion had to be greater than half a disc area.

Analysis of the primary outcome measure, change in GA size, revealed that at the study endpoint, there was a 20.4% reduction in mean change from baseline in

Table 2 Clinical trials targeting complement factor in Dry AMD

Molecule	Company	Clinical trial number	Status
Lampalizumab	Hoffmann-LaRoche (Basel, Switzerland)	NCT02247479	In phase 3
Zimura	Ophthotech (Princeton, New Jersey, USA)	NCT00950638	Completed phase 1
Eculizumab	Alexion Pharmaceuticals (Cheshire, Connecticut, USA)	NCT00935883	Completed phase 2

GA area with a *P*-value less than the prespecified significance level of 0.2. A positive treatment effect was observed starting at Month 6 and lasting until Month 18 using autofluorescence and color fundus photographs (http://www.roche.com/investors/updates/inv-update-2013-08-27.htm).

When a subgroup analysis was performed on patients who were noted to be positive for exploratory biomarkers (mutations for CFH, C3, C2/CFB, and complement factor I), the treatment group was found to have a 44% reduction at the study's conclusion compared to the sham group ($P < 0.005$ with $N = 28$ at the final time point). Within the pooled sham group, the complement factor I mutation group had significantly more atrophy than the factor I negative group. The study authors conclude that the treatment response was magnified in patients with the complement factor I biomarker, which acts downstream of factor D and CFH in the alternative complement pathway. When analyzing all patients, final BCVA appeared worse in all groups compared to baseline. The authors concluded that as vision was similarly worse in the sham versus the treatment groups, the drug itself was not implicated as a cause of acuity changes.

Zimura (ARC-1905) is a PEGylated nucleic acid aptamer that targets and inhibits complement factor C5 by blocking the cleavage of C5 into C5a and C5b fragments (http://www.ophthotech.com/product-candidates/). Phase I trial for dry AMD evaluated the safety and tolerability of intravitreous Zimura injection in patients with GA secondary to dry AMD. The results have not been posted, but Ophthotech plans to initiate Phase II/III clinical trial (http://www.ophthotech.com/product-candidates/).

The complement inhibition with eculizumab for the treatment of nonexudative AMD (COMPLETE) Phase II trial on eculizumab recruited 60 participants (50 years and older) with dry AMD. The study showed that systemic complement inhibition with eculizumab was well tolerated through 6 months, but did not decrease the growth rate of GA or drusen significantly (Table 2) (Yehoshua et al. 2014; Garcia Filho et al. 2014).

References

Antonetti DA, Wolpert EB, DeMaio L, Harhaj NS, Scaduto RC Jr (2002) Hydrocortisone decreases retinal endothelial cell water and solute flux coincident with increased content and decreased phosphorylation of occludin. J Neurochem 80(4):667–677

Boyer DS, Faber D, Gupta S, Patel SS, Tabandeh H, Li XY et al (2011) Dexamethasone intravitreal implant for treatment of diabetic macular edema in vitrectomized patients. Retina 31(5):915–923

Boyer DS, Yoon YH, Belfort R Jr, Bandello F, Maturi RK, Augustin AJ et al (2014) Three-year, randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with diabetic macular edema. Ophthalmology 121(10):1904–1914

Campochiaro PA, Brown DM, Pearson A, Chen S, Boyer D, Ruiz-Moreno J et al (2012) Sustained delivery fluocinolone acetonide vitreous inserts provide benefit for at least 3 years in patients with diabetic macular edema. Ophthalmology 119(10):2125–2132

Chang-Lin JE, Burke JA, Peng Q, Lin T, Orilla WC, Ghosn CR et al (2011) Pharmacokinetics of a sustained-release dexamethasone intravitreal implant in vitrectomized and nonvitrectomized eyes. Invest Ophthalmol Vis Sci 52(7):4605–4609

Diabetic Retinopathy Clinical Research Network (2008) A randomized trial comparing intravitreal triamcinolone acetonide and focal/grid photocoagulation for diabetic macular edema. Ophthalmology 115(9):1447–9, 1449.e1–10

Diabetic Retinopathy Clinical Research Network, Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB et al (2010) Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 117 (6):1064–1077.e35

Ding X, Patel M, Chan CC (2009) Molecular pathology of age-related macular degeneration. Prog Retin Eye Res 28(1):1–18

Elman MJ, Bressler NM, Qin H, Beck RW, Ferris FL 3rd, Friedman SM et al (2011) Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 118(4):609–614

Felinski EA, Antonetti DA (2005) Glucocorticoid regulation of endothelial cell tight junction gene expression: novel treatments for diabetic retinopathy. Curr Eye Res 30(11):949–957

Garcia Filho CA, Yehoshua Z, Gregori G, Nunes RP, Penha FM, Moshfeghi AA et al (2014) Change in drusen volume as a novel clinical trial endpoint for the study of complement inhibition in age-related macular degeneration. Ophthalmic Surg Lasers Imaging Retina 45 (1):18–31

Gillies MC, Lim LL, Campain A, Quin GJ, Salem W, Li J et al (2014) A randomized clinical trial of intravitreal bevacizumab versus intravitreal dexamethasone for diabetic macular edema: the BEVORDEX study. Ophthalmology 121(12):2473–2481

Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 20(6):705–732

Haller JA, Bandello F, Belfort R Jr, Blumenkranz MS, Gillies M, Heier J et al (2010) Randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with macular edema due to retinal vein occlusion. Ophthalmology 117(6):1134–1146.e3

Hashizume K, Hirasawa M, Imamura Y, Noda S, Shimizu T, Shinoda K et al (2008) Retinal dysfunction and progressive retinal cell death in SOD1-deficient mice. Am J Pathol 172 (5):1325–1331

http://www.ophthotech.com/product-candidates/. Accessed 18 April 2016

http://www.roche.com/investors/updates/inv-update-2013-08-27.htm. Accessed 18 April 2016

Ip MS, Scott IU, VanVeldhuisen PC, Oden NL, Blodi BA, Fisher M et al (2009) A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5. Arch Ophthalmol 127(9):1101–1114

Kuppermann BD, Goldstein M, Maturi RK, Pollack A, Singer M, Tufail A et al (2015) Dexamethasone intravitreal implant as adjunctive therapy to ranibizumab in neovascular age-related

macular degeneration: a multicenter randomized controlled trial. Ophthalmologica 234 (1):40–54

Lee WJ, Kang MH, Seong M, Cho HY (2012) Comparison of aqueous concentrations of angiogenic and inflammatory cytokines in diabetic macular oedema and macular oedema due to branch retinal vein occlusion. Br J Ophthalmol 96(11):1426–1430

Maturi RK, Bleau L, Saunders J, Mubasher M, Stewart MW (2015) A 12-month, single-masked, randomized controlled study of eyes with persistent diabetic macular edema after multiple anti-VEGF injections to assess the efficacy of the dexamethasone-delayed delivery system as an adjunct to bevacizumab compared with continued bevacizumab monotherapy. Retina 35 (8):1604–1614

Noma H, Funatsu H, Yamasaki M, Tsukamoto H, Mimura T, Sone T et al (2005) Pathogenesis of macular edema with branch retinal vein occlusion and intraocular levels of vascular endothelial growth factor and interleukin-6. Am J Ophthalmol 140(2):256–261

Ozaki E, Campbell M, Kiang AS, Humphries M, Doyle SL, Humphries P (2014) Inflammation in age-related macular degeneration. Adv Exp Med Biol 801:229–235

Ranchod TM, Ray SK, Daniels SA, Leong CJ, Ting TD, Verne AZ (2013) LuceDex: a prospective study comparing ranibizumab plus dexamethasone combination therapy versus ranibizumab monotherapy for neovascular age-related macular degeneration. Retina 33(8):1600–1604

Scott IU, Ip MS, VanVeldhuisen PC, Oden NL, Blodi BA, Fisher M et al (2009) A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular Edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6. Arch Ophthalmol 127(9):1115–1128

Tamura H, Miyamoto K, Kiryu J, Miyahara S, Katsuta H, Hirose F et al (2005) Intravitreal injection of corticosteroid attenuates leukostasis and vascular leakage in experimental diabetic retina. Invest Ophthalmol Vis Sci 46(4):1440–1444

Tang J, Kern TS (2011) Inflammation in diabetic retinopathy. Prog Retin Eye Res 30(5):343–358

Whitcup SM, Sodhi A, Atkinson JP, Holers VM, Sinha D, Rohrer B et al (2013a) The role of the immune response in age-related macular degeneration. Int J Inflamm 2013:348092

Whitcup SM, Nussenblatt RB, Lightman SL, Hollander DA (2013b) Inflammation in retinal disease. Int J Inflamm 2013:724648

Xu H, Chen M, Forrester JV (2009) Para-inflammation in the aging retina. Prog Retin Eye Res 28 (5):348–368

Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, Gregori G, Penha FM, Moshfeghi AA et al (2014) Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. Ophthalmology 121(3):693–701

Zhao Z, Chen Y, Wang J, Sternberg P, Freeman ML, Grossniklaus HE et al (2011) Age-related retinopathy in NRF2-deficient mice. PLoS One 6(4):e19456

Zhioua I, Semoun O, Lalloum F, Souied EH (2015) Intravitreal dexamethasone implant in patients with ranibizumab persistent diabetic macular edema. Retina 35(7):1429–1435

Dry Age-Related Macular Degeneration Pharmacology

Charles B. Wright and Jayakrishna Ambati

Contents

Abstract

Age-related macular degeneration (AMD), the most common form of irreversible blindness in the industrially developed world, can present years before a patient begins to lose vision. For most of these patients, AMD never progresses past its early stages to the advanced forms that are principally responsible for the vast majority of vision loss. Advanced AMD can manifest as either an advanced avascular form known as geographic atrophy (GA) marked by regional retinal pigment epithelium (RPE) cell death or as an advanced form known as neovascular AMD marked by the intrusion of fragile new blood vessels into the normally avascular retina. Physicians have several therapeutic interventions available to combat neovascular AMD, but GA has no approved effective therapies as of yet. In this chapter, we will discuss the current strategies for limiting

C.B. Wright • J. Ambati (✉)
Physiology and Ophthalmology and Visual Sciences, University of Kentucky College of Medicine, Lexington, KY 40506, USA
e-mail: JA9QR@hscmail.mcc.virginia.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_36

dry AMD in patients. We will also discuss previous attempts at pharmacological intervention that were tested in a clinical setting and consider reasons why these putative therapeutics did not perform successfully in large-scale trials. Despite the number of unsuccessful past trials, new pharmacological interventions may succeed. These future therapies may aid millions of AMD patients worldwide.

Keywords
Age-related macular degeneration • Experimental medicine • Ocular pharmacology

1 Introduction

Age-related macular degeneration (AMD) is the most prevalent cause of blindness in the elderly in the USA (Schmier et al. 2012) and affects as many as one in eight individuals over the age of 80 (Zeng et al. 2016). AMD affects many different cell types, including the retinal pigment epithelium (RPE) (Ambati and Fowler 2012), choriocapillaris endothelial cells (ECs) (Zeng et al. 2016), and photoreceptor cells (Carr et al. 2013). Early AMD initially presents with drusen, whitish or yellowish punctate extracellular deposits >63 μm in diameter positioned between the RPE and Bruch's membrane (Ferris et al. 2013). Changes to RPE pigmentation mark the transition to intermediate AMD (Ratnapriya and Chew 2013). Noticeable declines in visual function do not occur until advanced forms of AMD, which may take years (Buschini et al. 2015). Advanced AMD takes two primary forms defined by whether neovascularization occurs; geographic atrophy (GA) (Buschini et al. 2015) presents with regional RPE cell death without blood vessel intrusion, while exudative (i.e., wet) AMD does (Wong et al. 2008). Over three-quarters of legal blindness results from wet AMD (Buschini et al. 2015), but GA accounts for the vast majority of advanced AMD cases (Tarallo et al. 2012). Unfortunately, no therapeutic options for dry AMD are available (Tarallo et al. 2012).

Because age is the greatest risk factor for AMD (Bora et al. 2014) and the US census data indicates the number of elderly individuals is expected to greatly increase in the next few decades, the number of patients with AMD is expected to almost double by the year 2050 (Rein et al. 2009). Given the large number of patients with untreatable dry AMD and the reduced quality of life experienced by these patients, there is an immediate need for effective medications to treat the disease. To date, no potential therapeutics show efficacy with respect to slowing or reversing dry AMD progression. The purpose of this chapter is to explore the different therapeutic targets and their proposed treatments and to consider reasons for why these approaches have not been effective.

2 Current Strategies for Addressing Dry AMD

Lifestyle modification and dietary supplements are the only options to combat AMD development, progression, and associated visual function decline. Modifiable risk factors for dry AMD include smoking and obesity (Cheung and Eaton 2013). Smoking in particular has been associated with development and progression of AMD (Buschini et al. 2015), and some evidence AMD risk has a dose-dependent relationship with smoking (Velilla et al. 2013). Analysis of pooled datasets from the Beaver Dam Eye Study (Klein et al. 1993), Rotterdam Study (Vingerling et al. 1995), and Blue Mountains Eye Study (Mitchell et al. 1995) suggests smokers have an approximately three-fold greater risk of AMD than patients who never smoked (Smith et al. 2001). Patients who quit smoking greatly reduce their risk for developing AMD, but are still at a greater risk for AMD than patients who never smoked (Velilla et al. 2013; Smith et al. 2001). Much of the RPE toxicity has been attributed to reactive oxygen species (ROS) formation (Woodell and Rohrer 2014). Mice exposed to cigarette smoke exhibit signs of oxidative damage that recapitulate some symptoms of AMD, such as Bruch's membrane thickening, basal infoldings in the RPE, and RPE cell death (Fujihara et al. 2008). Smoking also promotes the formation of advanced glycation end-products (AGEs) (Kirkham et al. 2003) and deposition of cadmium, which promotes ROS production, in the RPE (Woodell and Rohrer 2014; Kirkham et al. 2003). Like smoking, obesity and high-fat diets have also been associated with early AMD and progression to late AMD (Cheung and Eaton 2013). The Beaver Dam Eye Study, for example, suggested a potential link between obesity and AMD (Howard et al. 2014). High glucose (Ghaem Maralani et al. 2015), high triglycerides (Ghaem Maralani et al. 2015), and daily red meat consumption (Ersoy et al. 2014) all appear to contribute to AMD risk. Collectively, these findings suggest lifestyle modifications may reduce the risk of AMD.

Because observations indicated diets rich in fruits and vegetables may protect against AMD (Ersoy et al. 2014; Seddon et al. 1994), the Age-Related Eye Disease Study (AREDS) (Age-Related Eye Disease Study Research 2001) was conducted to determine whether a dietary supplement containing high-dose vitamins C and E, beta carotene, and zinc could protect against AMD progression by reducing oxidative stress (Cheung and Eaton 2013). A second study, AREDS2, tested lutein, zeaxanthin, and omega-3 fatty acids for AMD protection (Age-Related Eye Disease Study 2 Research G 2013). An initial report suggested the supplement slowed AMD progression (Age-Related Eye Disease Study Research 2001), but closer examination of the data suggested that it was ineffective (Ambati and Ambati 2002). Indeed, a meta-analysis of multiple AREDS trials indicated the supplement was ineffective (Evans 2008), a finding supported by follow-up examination of the original AREDS study participants (Chew et al. 2014). At the moment, lifestyle modification may be the only option to prevent the development and progression of AMD.

3 Previous Pharmacological Interventions

To date, there are no approved therapies effective at treating dry AMD despite decades of intensive research. Unlike neovascular AMD (Cheung and Eaton 2013; Schmidt-Erfurth et al. 2014), dry AMD continues to defy searches for a therapeutic target for intervention (Cheung and Eaton 2013). This section of the chapter will review both previous and current therapeutic interventions being tested for dry AMD treatment and the cell signaling pathways targeted by those interventions.

3.1 Antioxidants, Vitamins, and Herbal Supplements

Oxidative and mitochondrial stress may promote AMD development and progression (Hollyfield et al. 2008; Jarrett and Boulton 2012; Barot et al. 2011; Liang and Godley 2003), but to date, there are no effective therapies targeting oxidative damage or cellular oxidative response pathways. The AREDS dietary supplement formulations do not appear to be effective in preventing AMD progression (Age-Related Eye Disease Study Research G 2001; Evans 2008; Chew et al. 2014). Other herbal supplements and vitamin formulations not related to the AREDS and AREDS2 studies are also being studied for any potential efficacy against AMD progression. Some studies suggest *Ginkgo biloba* extract may protect against AMD by modulating choroidal blood flow and scavenging free radicals (Wilkinson and Fraunfelder 2011), and small trials conducted in Germany and France suggest the supplement may preserve vision in patients (Evans 2013). No large-scale clinical trials studying *G. biloba* extract have been conducted, however. Similarly, curcumin may inhibit the formation of oxidized lipids in oxidative stress conditions (Mandal et al. 2009), but no clinical trials have been performed yet. Resveratrol is also of interest because of its antioxidant activity (Pervaiz and Holme 2009), but given its limited testing in a clinical setting (Richer et al. 2014), results are inconclusive pending further investigation.

Other pharmacological interventions targeting the oxidative stress response include 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) agonists (Collier et al. 2011; Jaffe et al. 2015) and OT-551 (Wong et al. 2010). The 5-HT$_{1A}$ receptor is best known for its role in mediating serotonin-dependent signaling events involved in regulating sleep and anxiety, but receptor activation has also been demonstrated to protect against oxidative stress-induced RPE and photoreceptor cell death (Collier et al. 2011). The 5-HT$_{1A}$ agonists 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) and tandospirone (AL-8309A) protect against light-induced retinal damage in rodents (Collier et al. 2011; Biswal et al. 2015) by mitigating oxidative damage to mitochondria (Biswal et al. 2015). Unfortunately, tandospirone was not found to prevent lesion growth in GA patients during a phase III trial (Jaffe et al. 2015), casting doubt on the potential utility of 5-HT$_{1A}$ agonists in treating dry AMD. Similarly, OT-551 (Evans and Syed 2013) appeared promising in preliminary studies but failed to significantly halt the GA lesion spread in a phase II clinical trial (Wong et al. 2010).

3.2 Visual Cycle Modulators

Analysis of eyecups obtained from AMD patients found increased amounts of N-retinyl-N-retinylidene ethanolamine (A2E) compared to eyecups obtained from healthy patients (Suter et al. 2000). Two molecules of all-*trans*-retinal (Redmond et al. 1998) react with ethanolamine in oxidative conditions to produce A2E (Suter et al. 2000), which accumulates in the RPE with age (Eldred 1995). A2E appeared to induce RPE toxicity by suppressing lysosomal function, suggesting visual cycle byproducts may contribute to AMD pathology (Suter et al. 2000). Because A2E is toxic to RPE and was thought to be a principal component of the autofluorescent material lipofuscin that appears in many AMD patients (Eldred 1995), several groups attempted to create therapeutics that could halt its formation and slow AMD progression.

Given the well-defined functional and biochemical role of the visual cycle protein RPE-specific protein 65 kDa (RPE65) (Redmond et al. 1998), inhibitors specific to RPE65 were developed to slow the rate of retinoid metabolism to slow A2E generation (Buschini et al. 2015; Zhang et al. 2015). Emixustat specifically binds RPE65 at its active site to inhibit its activity (Kubota et al. 2014). Later work found emixustat scavenges free retinoids as well, and that this activity heavily contributes to its mechanism-of-action (Zhang et al. 2015). Early phase I clinical trials tested the safety and tolerance of emixustat through oral administration of the drug in doses ranging from 5 to 40 mg over a 14-day period (Kubota et al. 2014). Two-thirds of patients experienced mild adverse reactions to the drug, and because these adverse reactions resolved at the end of the study (Kubota et al. 2014), emixustat proceeded to phase II/III clinical trial (Dugel et al. 2015). This clinical trial is expected to be completed by July 2016.

Fenretinide (N-4-hydroxyphenylretinamide) also targets retinoid metabolism to slow AMD progression. The synthetic retinoid was originally designed as a potential therapeutic to use against various cancers that require retinoids for tumorigenesis, but was found to be ineffective against cancer progression in phase II/III clinical trials (Malone et al. 2003). Similarly to emixustat, fenretinide reduces the total pool of vitamin A-derived retinoids needed to form A2E (Danis et al. 2015). Mechanistically, fenretinide binds to retinol-binding protein 4 (RBP4) in the serum to interfere with vitamin A transport to various tissues (Petrukhin 2013), effectively causing vitamin A to be cleared through the urine (Danis et al. 2015). Unfortunately, phase II clinical trial data indicated fenretinide did not significantly reduce the rate of lesion expansion in GA patients (Mata et al. 2013). There are no known plans to carry out a phase III clinical trial of fenretinide as a GA therapeutic agent (Danis et al. 2015). Other nonretinoid RBP4 antagonists (Cioffi et al. 2015) such as A1120 are also currently being tested for efficacy against AMD-like phenotypes in mouse models with high rates of lipofuscinogenesis (Petrukhin 2013; Dobri et al. 2013), but these studies have not yet been translated to human patients.

3.3 Inflammatory Modulators

With the proliferation of studies suggesting pro-inflammatory pathways may be involved in AMD, anti-inflammatory agents are now of particular interest. A 1992 study reported elevated amounts of complement proteins C1q, C3c, and C3d in subretinal membranes removed from AMD patients (Baudouin et al. 1992). A series of landmark studies also identified the complement factor H Y402H (CFH^{Y402H}) polymorphism as the first heritable risk factor for AMD (Haines et al. 2005; Edwards et al. 2005; Klein et al. 2005), further highlighting the potential role of the complement cascade pathway in AMD pathogenesis. Unfortunately, the identification of both complement factor polymorphisms and complement factor proteins in AMD patients have not successfully facilitated the development of AMD therapeutics. The COMPLETE study (Yehoshua et al. 2014), which tested eculizumab, an anti-C5 antibody already approved for use in paroxysomal nocturnal hemoglobinuria (Buschini et al. 2015), found complement cascade inhibition had no effect on GA progression (Yehoshua et al. 2014). Similarly, the anti-C5 antibody LFG316 was ineffective in slowing or halting GA progression. Clinical trial data for another anti-C5 antibody, ARC1905, has not been published (Buschini et al. 2015). A neutralizing antibody targeting complement factor D (CFD) in GA patients, lampalizumab, is now in phase III trials (Danis et al. 2015).

Sirolimus, also known as rapamycin, has known anti-inflammatory properties (Mata and Vogel 2010). Already used as an immunosuppressant for organ transplant patients (Danis et al. 2015), it was hypothesized sirolimus may be effective in treating AMD as well because it was found to prevent RPE cell death in mouse models (Zhao et al. 2011). Two phase II clinical trials found no protective effect of sirolimus against GA, with no reported visual acuity protection or prevention of atrophic lesion spreading (Wong et al. 2013; Petrou et al. 2015).

Other efforts to treat AMD center on the NLRP3 inflammasome. A series of studies published within the last five years indicate DICER1 reduction in GA patients may contribute to RPE cell death because the enzyme is required to degrade cytotoxic *Alu* RNA transcripts (Kaneko et al. 2011). Increases in *Alu* RNA transcripts in GA patients cause activation of the NLRP3 inflammasome (Tarallo et al. 2012; Dridi et al. 2012), ultimately resulting in P2X7-dependent Caspase-8-mediated RPE cell death (Kerur et al. 2013; Kim et al. 2014). These observations are supported by other data showing this signaling pathway can be instigated by the presence of excess iron (Gelfand et al. 2015), which has also been previously associated with AMD in humans (Wong et al. 2007). It was recently found that nucleoside reverse transcriptase inhibitors (NRTIs), typically used to treat human immunodeficiency virus (HIV) patients, possessed anti-inflammatory properties because of their ability to block P2X7-dependent NLRP3 inflammasome activation (Wong et al. 2007). Preparations are currently underway to examine NRTIs in an AMD context in a clinical setting.

3.4 Neuroprotective Agents

Given the fact that blindness in GA is the direct result of photoreceptor death over regions with RPE atrophy (Danis et al. 2015), neuroprotective agents preventing photoreceptor cell apoptosis have been proposed as potential therapeutics. Ciliary neurotrophic factor (CNTF), for example, was previously demonstrated to preserve photoreceptor cell function and reduce apoptosis in various models of canine (Tao et al. 2002) and mouse retinal degeneration (LaVail et al. 1998). An encapsulated cell therapy (ECT)-based implant housing mammalian cells engineered to overproduce CNTF (Thanos et al. 2004) was injected into the eyes of GA patients in phase I (Sieving et al. 2006) and II clinical trials (Zhang et al. 2011). The CNTF-producing implant, NT-501 (Thanos et al. 2004), was found to be well-tolerated in patients (Sieving et al. 2006), but could not inhibit lesion spreading in GA patients (Zhang et al. 2011).

More recently, various groups have begun exploring the potential role of amyloid β in AMD. Amyloid β, perhaps best known for its suspected role in Alzheimer's disease (Kang et al. 1987; Hardy and Higgins 1992; Hardy and Selkoe 2002), was found to be a drusen component in AMD patients (Johnson et al. 2002; Dentchev et al. 2003). Ocular amyloid deposits in a mouse model of Alzheimer's disease were associated with retinal degeneration (Ning et al. 2008) and RPE stress (Ding et al. 2011), and amyloid β is known to activate the NLRP3 inflammasome (Halle et al. 2008), consistent with other findings that inflammasome activation may mediate GA (Tarallo et al. 2012; Kerur et al. 2013; Kim et al. 2014; Gelfand et al. 2015). Based on the studies suggesting amyloid β accumulation may contribute to ocular pathologies in mouse models of AMD (Ding et al. 2011; Catchpole et al. 2013), a clinical trial testing the efficacy of a monoclonal antibody against amyloid β is currently underway (Danis et al. 2015). The results of the phase II trial are not yet available.

3.5 Cell-Based Therapies

With the advent of human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) technologies, recent therapeutic efforts focus on transplanting healthy derived RPE cells into GA patients (Danis et al. 2015). The first hESCs implanted into humans occurred in 2012 when hESCs were differentiated into RPE cells for subretinal implantation in patients with either Stargardt's disease or dry AMD (Schwartz et al. 2012). Following transplantation, patients were administered immunosuppressive drugs to prevent tissue rejection, and the patients were closely monitored for complications (Schwartz et al. 2012). Preliminary data indicate the therapy may be safe in human patients, but visual acuity improvements appeared marginal (Schwartz et al. 2012). A follow-up phase I/II clinical trial indicated a majority of patients who received RPE transplantation showed small regions of pigmentation several months following treatment, suggesting derived RPE cells injected into the eyes of patients could form a partial monolayer (Schwartz et al. 2015). Although the authors of the study claim visual acuity improvements

in eyes receiving treatment compared to control eyes (Schwartz et al. 2015), the small patient sample is not currently sufficient to allow any definitive conclusions on whether hESC-derived RPE cells will effectively restore meaningful vision in AMD patients. The relatively small number of RPE cells surviving transplantation several months following treatments also suggests more refined methods of implantation using improved monolayers may be needed for these treatments to maximize their efficacy (Brandl et al. 2015). iPSC-derived RPE cells may offer advantages over hESC-derived RPE cells with respect to long-term safety. Because iPSC-derived RPE cells allow for autologous transplantation, future patients will hopefully have less risk of transplant rejection and obviate the need for immunosuppressive drugs (Brandl et al. 2015). Clinical trials for iPSC-derived RPE cells are already underway, and a case study of a woman who received iPSC-derived RPE cell transplantation suggests they may be as safe as hESC-derived RPE cells (Forest et al. 2015). The results of these clinical trials will be forthcoming once they are complete.

4 Perspectives on Future Directions

Numerous lines of evidence suggest the field has long suffered from gaps in understanding with respect to basic AMD biology. The inability of the visual cycle therapeutic fenretinide to halt GA lesion growth (Danis et al. 2015; Mata et al. 2013), for example, supports this hypothesis. Fenretinide successfully depletes vitamin A in the serum (Danis et al. 2015) but has no effect on disease progression in humans. A primary reason fenretinide failed in clinical trials could be the fact that the field overestimated the importance of A2E in AMD pathogenesis. It was originally thought A2E correlated with AMD (Suter et al. 2000), but later work found A2E primarily accumulated in the periphery of AMD eyes (Bhosale et al. 2009), not in the macula. Furthermore, later work also demonstrated that lipofuscin only poorly predicted lesion spread in GA (Hwang et al. 2006) and that the presence of A2E and lipofuscin did not even correlate with one another (Ablonczy et al. 2013). Together, these data indicate A2E might be largely irrelevant to AMD and that visual cycle therapeutics like fenretinide would have little chance of having a protective effect in patients. Similarly, numerous clinical trials focused on targeting complement factors to prevent GA spread but were ultimately found ineffective. The biological relevance of CFH polymorphisms in and AMD context (Haines et al. 2005; Edwards et al. 2005; Klein et al. 2005) remains elusive; *Cfh* mutant mice show only a marginal phenotype (Coffey et al. 2007), and many individuals exhibiting complement deposition in the RPE never present with AMD (Anderson et al. 2010; Mullins et al. 2014). Thus, the field may be missing the biological understanding of AMD needed to create effective therapeutics.

Inadequate experimental design and data interpretation also confound efforts to treat dry AMD. The AREDS study, for example, originally reported the AREDS vitamin supplement could significantly slow the progression of AMD from its earlier to its more advanced stages, but further data analysis and later patient follow-up indicated AREDS was actually ineffective (Ambati and Ambati 2002; Evans 2008; Chew et al. 2014). Similarly, the role of the NLRP3 inflammasome

Table 1 Therapeutics tested in the clinic for dry AMD

Agent	Intervention	Targeted biology
AREDS supplement	Dietary supplement	Oxidative stress
G. biloba extract	Dietary supplement	Oxidative stress
Curcumin	Dietary supplement	Oxidative stress
Resveratrol	Dietary supplement	Oxidative stress
8-OH-DPAT	Small molecule	Oxidative stress
Tandospirone (AL-8309A)	Small molecule	Oxidative stress
OT-551	Small molecule	Oxidative stress
Emixustat[a]	Small molecule	Visual cycle
Fenretinide	Small molecule	Visual cycle
A1120	Small molecule	Visual cycle
Eculizumab	Antibody	Complement cascade
LFG316	Antibody	Complement cascade
ARC1905	Antibody	Complement cascade
Lampalizumab[a]	Antibody	Complement cascade
Rapamycin	Small molecule	Inflammation (mTOR signaling)
CNTF	Recombinant protein	Neuroprotection
GSK 933776[a]	Antibody	Amyloid-β aggregation
iPSC-derived RPE[a]	Cell-based therapy	RPE loss

[a]Clinical trials have not yet been completed

with respect to AMD was at first unclear because of experimental design issues. After the NLRP3 inflammasome was linked to GA (Tarallo et al. 2012), the finding was challenged because of data demonstrating IL-18 antibody neutralization augmented laser-induced choroidal neovascularization in mice (Doyle et al. 2012). It was later found the IL-18 neutralizing antibody solution injected into mice contained glycerol, which is toxic to the retina and RPE; moreover, other laboratories could not replicate findings suggesting the NLRP3 inflammasome was protective (Ijima et al. 2014; Zhang et al. 2016; Wang et al. 2016; Tseng et al. 2013; Kauppinen et al. 2012; Hirano et al. 2014). In order for effective therapeutic options to become available to patients with dry AMD, the field requires a better understanding of the basic biology underpinning disease pathogenesis, which in turn requires rigorous experimental design and analysis.

5 Conclusions

The complexity of AMD pathogenesis continues to stymie the development of effective pharmacological interventions for the disease. Unlike neovascular AMD, which has a number of therapeutic options for patients, dry AMD currently has no effective pharmacological intervention. Previous attempts to slow disease progression have been unsuccessful, but a new generation of novel pharmacological approaches and cell-based therapies may have a greater degree of success in treating the disease (Table 1).

References

Ablonczy Z, Higbee D, Anderson DM, Dahrouj M, Grey AC, Gutierrez D, Koutalos Y, Schey KL, Hanneken A, Crouch RK (2013) Lack of correlation between the spatial distribution of A2E and lipofuscin fluorescence in the human retinal pigment epithelium. Investig Ophthalmol Vis Sci 54(8):5535–5542. doi:10.1167/iovs.13-12250

Age-Related Eye Disease Study 2 Research G (2013) Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. JAMA 309(19):2005–2015. doi:10.1001/jama.2013.4997

Age-Related Eye Disease Study Research G (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 119(10):1417–1436

Ambati J, Ambati BK (2002) Age-related eye disease study caveats. Arch Ophthalmol 120(7):997, author reply 997–999

Ambati J, Fowler BJ (2012) Mechanisms of age-related macular degeneration. Neuron 75(1): 26–39. doi:10.1016/j.neuron.2012.06.018

Anderson DH, Radeke MJ, Gallo NB, Chapin EA, Johnson PT, Curletti CR, Hancox LS, Hu J, Ebright JN, Malek G, Hauser MA, Rickman CB, Bok D, Hageman GS, Johnson LV (2010) The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. Prog Retin Eye Res 29(2):95–112. doi:10.1016/j.preteyeres.2009.11.003

Barot M, Gokulgandhi MR, Mitra AK (2011) Mitochondrial dysfunction in retinal diseases. Curr Eye Res 36(12):1069–1077. doi:10.3109/02713683.2011.607536

Baudouin C, Peyman GA, Fredj-Reygrobellet D, Gordon WC, Lapalus P, Gastaud P, Bazan NG (1992) Immunohistological study of subretinal membranes in age-related macular degeneration. Jpn J Ophthalmol 36(4):443–451

Bhosale P, Serban B, Bernstein PS (2009) Retinal carotenoids can attenuate formation of A2E in the retinal pigment epithelium. Arch Biochem Biophys 483(2):175–181. doi:10.1016/j.abb.2008.09.012

Biswal MR, Ahmed CM, Ildefonso CJ, Han P, Li H, Jivanji H, Mao H, Lewin AS (2015) Systemic treatment with a 5HT1a agonist induces anti-oxidant protection and preserves the retina from mitochondrial oxidative stress. Exp Eye Res 140:94–105. doi:10.1016/j.exer.2015.07.022

Bora NS, Matta B, Lyzogubov VV, Bora PS (2014) Relationship between the complement system, risk factors and prediction models in age-related macular degeneration. Mol Immunol. doi:10.1016/j.molimm.2014.07.012

Brandl C, Grassmann F, Riolfi J, Weber BH (2015) Tapping stem cells to target AMD: challenges and prospects. J Clin Med 4(2):282–303. doi:10.3390/jcm4020282

Buschini E, Fea AM, Lavia CA, Nassisi M, Pignata G, Zola M, Grignolo FM (2015) Recent developments in the management of dry age-related macular degeneration. Clin Ophthalmol 9:563–574. doi:10.2147/OPTH.S59724

Carr AJ, Smart MJ, Ramsden CM, Powner MB, da Cruz L, Coffey PJ (2013) Development of human embryonic stem cell therapies for age-related macular degeneration. Trends Neurosci 36(7):385–395. doi:10.1016/j.tins.2013.03.006

Catchpole I, Germaschewski V, Hoh Kam J, Lundh von Leithner P, Ford S, Gough G, Adamson P, Overend P, Hilpert J, Lopez FJ, Ng YS, Coffey P, Jeffery G (2013) Systemic administration of Abeta mAb reduces retinal deposition of Abeta and activated complement C3 in age-related macular degeneration mouse model. PLoS One 8(6), e65518. doi:10.1371/journal.pone.0065518

Cheung LK, Eaton A (2013) Age-related macular degeneration. Pharmacotherapy 33(8):838–855. doi:10.1002/phar.1264

Chew EY, Clemons TE, Agron E, Sperduto RD, Sangiovanni JP, Davis MD, Ferris FL 3rd, Age-Related Eye Disease Study Research G (2014) Ten-year follow-up of age-related macular degeneration in the age-related eye disease study: AREDS report no. 36. JAMA Ophthalmol 132(3):272–277. doi:10.1001/jamaophthalmol.2013.6636

Cioffi CL, Racz B, Freeman EE, Conlon MP, Chen P, Stafford DG, Schwarz DM, Zhu L, Kitchen DB, Barnes KD, Dobri N, Michelotti E, Cywin CL, Martin WH, Pearson PG, Johnson G, Petrukhin K (2015) Bicyclic [3.3.0]-Octahydrocyclopenta[c]pyrrolo Antagonists of Retinol Binding Protein 4: Potential Treatment of Atrophic Age-Related Macular Degeneration and Stargardt Disease. J Med Chem 58(15):5863–5888. doi:10.1021/acs.jmedchem.5b00423

Coffey PJ, Gias C, McDermott CJ, Lundh P, Pickering MC, Sethi C, Bird A, Fitzke FW, Maass A, Chen LL, Holder GE, Luthert PJ, Salt TE, Moss SE, Greenwood J (2007) Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction. Proc Natl Acad Sci U S A 104(42):16651–16656. doi:10.1073/pnas.0705079104

Collier RJ, Patel Y, Martin EA, Dembinska O, Hellberg M, Krueger DS, Kapin MA, Romano C (2011) Agonists at the serotonin receptor (5-HT(1A)) protect the retina from severe photo-oxidative stress. Investig Ophthalmol Vis Sci 52(5):2118–2126. doi:10.1167/iovs.10-6304

Danis RP, Lavine JA, Domalpally A (2015) Geographic atrophy in patients with advanced dry age-related macular degeneration: current challenges and future prospects. Clin Ophthalmol 9: 2159–2174. doi:10.2147/OPTH.S92359

Dentchev T, Milam AH, Lee VM, Trojanowski JQ, Dunaief JL (2003) Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. Mol Vis 9:184–190

Ding J-D, Johnson LV, Herrmann R, Farsiu S, Smith SG, Groelle M, Mace BE, Sullivan P, Jamison JA, Kelly U, Harrabi O, Bollini SS, Dilley J, Kobayashi D, Kuang B, Li W, Pons J, Lin JC, Bowes Rickman C (2011) Anti-amyloid therapy protects against retinal pigmented epithelium damage and vision loss in a model of age-related macular degeneration. Proc Natl Acad Sci U S A 108(28):E279–E287. doi:10.1073/pnas.1100901108

Dobri N, Qin Q, Kong J, Yamamoto K, Liu Z, Moiseyev G, Ma JX, Allikmets R, Sparrow JR, Petrukhin K (2013) A1120, a nonretinoid RBP4 antagonist, inhibits formation of cytotoxic bis-retinoids in the animal model of enhanced retinal lipofuscinogenesis. Investig Ophthalmol Vis Sci 54(1):85–95. doi:10.1167/iovs.12-10050

Doyle SL, Campbell M, Ozaki E, Salomon RG, Mori A, Kenna PF, Farrar GJ, Kiang AS, Humphries MM, Lavelle EC, O'Neill LA, Hollyfield JG, Humphries P (2012) NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. Nat Med 18(5):791–798. doi:10.1038/nm.2717

Dridi S, Hirano Y, Tarallo V, Kim Y, Fowler BJ, Ambati BK, Bogdanovich S, Chiodo VA, Hauswirth WW, Kugel JF, Goodrich JA, Ponicsan SL, Hinton DR, Kleinman ME, Baffi JZ, Gelfand BD, Ambati J (2012) ERK1/2 activation is a therapeutic target in age-related macular degeneration. Proc Natl Acad Sci U S A 109(34):13781–13786. doi:10.1073/pnas.1206494109

Dugel PU, Novack RL, Csaky KG, Richmond PP, Birch DG, Kubota R (2015) Phase ii, randomized, placebo-controlled, 90-day study of emixustat hydrochloride in geographic atrophy associated with dry age-related macular degeneration. Retina 35(6):1173–1183. doi:10.1097/IAE.0000000000000606

Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA (2005) Complement factor H polymorphism and age-related macular degeneration. Science 308(5720):421–424. doi:10.1126/science.1110189

Eldred GE (1995) Lipofuscin fluorophore inhibits lysosomal protein degradation and may cause early stages of macular degeneration. Gerontology 41(Suppl 2):15–28

Ersoy L, Ristau T, Lechanteur YT, Hahn M, Hoyng CB, Kirchhof B, den Hollander AI, Fauser S (2014) Nutritional risk factors for age-related macular degeneration. BioMed Res Int 2014: 413150. doi:10.1155/2014/413150

Evans J (2008) Antioxidant supplements to prevent or slow down the progression of AMD: a systematic review and meta-analysis. Eye 22(6):751–760. doi:10.1038/eye.2008.100

Evans JR (2013) Ginkgo biloba extract for age-related macular degeneration. Cochrane Database Syst Rev 1:CD001775. doi:10.1002/14651858.CD001775.pub2

Evans JB, Syed BA (2013) New hope for dry AMD? Nat Rev Drug Discov 12(7):501–502. doi:10.1038/nrd4038

Ferris FL 3rd, Wilkinson CP, Bird A, Chakravarthy U, Chew E, Csaky K, Sadda SR, Beckman Initiative for Macular Research Classification C (2013) Clinical classification of age-related macular degeneration. Ophthalmology 120(4):844–851. doi:10.1016/j.ophtha.2012.10.036

Forest DL, Johnson LV, Clegg DO (2015) Cellular models and therapies for age-related macular degeneration. Dis Model Mech 8(5):421–427. doi:10.1242/dmm.017236

Fujihara M, Nagai N, Sussan TE, Biswal S, Handa JT (2008) Chronic cigarette smoke causes oxidative damage and apoptosis to retinal pigmented epithelial cells in mice. PLoS One 3(9), e3119. doi:10.1371/journal.pone.0003119

Gelfand BD, Wright CB, Kim Y, Yasuma T, Yasuma R, Li S, Fowler BJ, Bastos-Carvalho A, Kerur N, Uittenbogaard A, Han YS, Lou D, Kleinman ME, McDonald WH, Nunez G, Georgel P, Dunaief JL, Ambati J (2015) Iron toxicity in the retina requires Alu RNA and the NLRP3 inflammasome. Cell Rep 11(11):1686–1693. doi:10.1016/j.celrep.2015.05.023

Ghaem Maralani H, Tai BC, Wong TY, Tai ES, Li J, Wang JJ, Mitchell P (2015) Metabolic syndrome and risk of age-related macular degeneration. Retina 35(3):459–466. doi:10.1097/IAE.0000000000000338

Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA (2005) Complement factor H variant increases the risk of age-related macular degeneration. Science 308(5720):419–421. doi:10.1126/science.1110359

Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT (2008) The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol 9(8):857–865. doi:10.1038/ni.1636

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256(5054):184–185

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297(5580):353–356. doi:10.1126/science.1072994

Hirano Y, Yasuma T, Mizutani T, Fowler BJ, Tarallo V, Yasuma R, Kim Y, Bastos-Carvalho A, Kerur N, Gelfand BD, Bogdanovich S, He S, Zhang X, Nozaki M, Ijima R, Kaneko H, Ogura Y, Terasaki H, Nagai H, Haro I, Nunez G, Ambati BK, Hinton DR, Ambati J (2014) IL-18 is not therapeutic for neovascular age-related macular degeneration. Nat Med 20(12):1372–1375. doi:10.1038/nm.3671

Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL (2008) Oxidative damage-induced inflammation initiates age-related macular degeneration. Nat Med 14(2):194–198. doi:10.1038/nm1709

Howard KP, Klein BE, Lee KE, Klein R (2014) Measures of body shape and adiposity as related to incidence of age-related eye diseases: observations from the Beaver Dam Eye Study. Investig Ophthalmol Vis Sci 55(4):2592–2598. doi:10.1167/iovs.13-13763

Hwang JC, Chan JW, Chang S, Smith RT (2006) Predictive value of fundus autofluorescence for development of geographic atrophy in age-related macular degeneration. Investig Ophthalmol Vis Sci 47(6):2655–2661. doi:10.1167/iovs.05-1027

Ijima R, Kaneko H, Ye F, Nagasaka Y, Takayama K, Kataoka K, Kachi S, Iwase T, Terasaki H (2014) Interleukin-18 induces retinal pigment epithelium degeneration in mice. Investig Ophthalmol Vis Sci 55(10):6673–6678. doi:10.1167/iovs.14-15367

Jaffe GJ, Schmitz-Valckenberg S, Boyer D, Heier J, Wolf-Schnurrbusch U, Staurenghi G, Schmidt-Erfurth U, Holz FG (2015) Randomized trial to evaluate tandospirone in geographic atrophy secondary to age-related macular degeneration: The GATE Study. Am J Ophthalmol 160(6):1226–1234. doi:10.1016/j.ajo.2015.08.024

Jarrett SG, Boulton ME (2012) Consequences of oxidative stress in age-related macular degeneration. Mol Aspects Med 33(4):399–417. doi:10.1016/j.mam.2012.03.009

Johnson LV, Leitner WP, Rivest AJ, Staples MK, Radeke MJ, Anderson DH (2002) The Alzheimer's A beta -peptide is deposited at sites of complement activation in pathologic deposits associated with aging and age-related macular degeneration. Proc Natl Acad Sci U S A 99(18):11830–11835. doi:10.1073/pnas.192203399

Kaneko H, Dridi S, Tarallo V, Gelfand BD, Fowler BJ, Cho WG, Kleinman ME, Ponicsan SL, Hauswirth WW, Chiodo VA, Karikó K, Yoo JW, Lee D-K, Hadziahmetovic M, Song Y, Misra S, Chaudhuri G, Buaas FW, Braun RE, Hinton DR, Zhang Q, Grossniklaus HE, Provis JM, Madigan MC, Milam AH, Justice NL, Albuquerque RJC, Blandford AD, Bogdanovich S, Hirano Y, Witta J, Fuchs E, Littman DR, Ambati BK, Rudin CM, Chong MMW, Provost P, Kugel JF, Goodrich JA, Dunaief JL, Baffi JZ, Ambati J (2011) DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. Nature. doi:10.1038/nature09830

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325(6106):733–736. doi:10.1038/325733a0

Kauppinen A, Niskanen H, Suuronen T, Kinnunen K, Salminen A, Kaarniranta K (2012) Oxidative stress activates NLRP3 inflammasomes in ARPE-19 cells--implications for age-related macular degeneration (AMD). Immunol Lett 147(1-2):29–33. doi:10.1016/j.imlet.2012.05.005

Kerur N, Hirano Y, Tarallo V, Fowler BJ, Bastos-Carvalho A, Yasuma T, Yasuma R, Kim Y, Hinton DR, Kirschning CJ, Gelfand BD, Ambati J (2013) TLR-independent and P2X7-dependent signaling mediate Alu RNA-induced NLRP3 inflammasome activation in geographic atrophy. Investig Ophthalmol Vis Sci 54(12):7395–7401. doi:10.1167/iovs.13-12500

Kim Y, Tarallo V, Kerur N, Yasuma T, Gelfand BD, Bastos-Carvalho A, Hirano Y, Yasuma R, Mizutani T, Fowler BJ, Li S, Kaneko H, Bogdanovich S, Ambati BK, Hinton DR, Hauswirth WW, Hakem R, Wright C, Ambati J (2014) DICER1/Alu RNA dysmetabolism induces Caspase-8-mediated cell death in age-related macular degeneration. Proc Natl Acad Sci U S A 111(45):16082–16087. doi:10.1073/pnas.1403814111

Kirkham PA, Spooner G, Ffoulkes-Jones C, Calvez R (2003) Cigarette smoke triggers macrophage adhesion and activation: role of lipid peroxidation products and scavenger receptor. Free Radic Biol Med 35(7):697–710

Klein R, Klein BE, Franke T (1993) The relationship of cardiovascular disease and its risk factors to age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology 100(3):406–414

Klein RJ, Zeiss C, Chew EY, Tsai J-Y, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308(5720):385–389. doi:10.1126/science.1109557

Kubota R, Al-Fayoumi S, Mallikaarjun S, Patil S, Bavik C, Chandler JW (2014) Phase 1, dose-ranging study of emixustat hydrochloride (ACU-4429), a novel visual cycle modulator, in healthy volunteers. Retina 34(3):603–609. doi:10.1097/01.iae.0000434565.80060.f8

LaVail MM, Yasumura D, Matthes MT, Lau-Villacorta C, Unoki K, Sung CH, Steinberg RH (1998) Protection of mouse photoreceptors by survival factors in retinal degenerations. Investig Ophthalmol Vis Sci 39(3):592–602

Liang FQ, Godley BF (2003) Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. Exp Eye Res 76(4):397–403

Malone W, Perloff M, Crowell J, Sigman C, Higley H (2003) Fenretinide: a prototype cancer prevention drug. Expert Opin Investig Drugs 12(11):1829–1842. doi:10.1517/13543784.12.11.1829

Mandal MN, Patlolla JM, Zheng L, Agbaga MP, Tran JT, Wicker L, Kasus-Jacobi A, Elliott MH, Rao CV, Anderson RE (2009) Curcumin protects retinal cells from light-and oxidant stress-induced cell death. Free Radic Biol Med 46(5):672–679. doi:10.1016/j.freeradbiomed.2008.12.006

Mata NL, Vogel R (2010) Pharmacologic treatment of atrophic age-related macular degeneration. Curr Opin Ophthalmol 21(3):190–196. doi:10.1097/ICU.0b013e32833866c8

Mata NL, Lichter JB, Vogel R, Han Y, Bui TV, Singerman LJ (2013) Investigation of oral fenretinide for treatment of geographic atrophy in age-related macular degeneration. Retina 33(3):498–507. doi:10.1097/IAE.0b013e318265801d

Mitchell P, Smith W, Attebo K, Wang JJ (1995) Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology 102(10):1450–1460

Mullins RF, Schoo DP, Sohn EH, Flamme-Wiese MJ, Workamelahu G, Johnston RM, Wang K, Tucker BA, Stone EM (2014) The membrane attack complex in aging human choriocapillaris: relationship to macular degeneration and choroidal thinning. Am J Pathol 184(11):3142–3153. doi:10.1016/j.ajpath.2014.07.017

Ning A, Cui J, To E, Ashe KH, Matsubara J (2008) Amyloid-beta deposits lead to retinal degeneration in a mouse model of Alzheimer disease. Investig Ophthalmol Vis Sci 49(11):5136–5143. doi:10.1167/iovs.08-1849

Pervaiz S, Holme AL (2009) Resveratrol: its biologic targets and functional activity. Antioxid Redox Signal 11(11):2851–2897. doi:10.1089/ARS.2008.2412

Petrou PA, Cunningham D, Shimel K, Harrington M, Hammel K, Cukras CA, Ferris FL, Chew EY, Wong WT (2015) Intravitreal sirolimus for the treatment of geographic atrophy: results of a phase I/II clinical trial. Investig Ophthalmol Vis Sci 56(1):330–338. doi:10.1167/iovs.14-15877

Petrukhin K (2013) Pharmacological inhibition of lipofuscin accumulation in the retina as a therapeutic strategy for dry AMD treatment. Drug Discov Today Ther Strateg 10(1):e11–e20. doi:10.1016/j.ddstr.2013.05.004

Ratnapriya R, Chew EY (2013) Age-related macular degeneration-clinical review and genetics update. Clin Genet 84(2):160–166. doi:10.1111/cge.12206

Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, Goletz P, Ma JX, Crouch RK, Pfeifer K (1998) Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. Nat Genet 20(4):344–351

Rein DB, Wittenborn JS, Zhang X, Honeycutt AA, Lesesne SB, Saaddine J, Vision Health Cost-Effectiveness Study G (2009) Forecasting age-related macular degeneration through the year 2050: the potential impact of new treatments. Arch Ophthalmol 127(4):533–540. doi:10.1001/archophthalmol.2009.58

Richer S, Patel S, Sockanathan S, Ulanski LJ 2nd, Miller L, Podella C (2014) Resveratrol based oral nutritional supplement produces long-term beneficial effects on structure and visual function in human patients. Nutrients 6(10):4404–4420. doi:10.3390/nu6104404

Schmidt-Erfurth U, Chong V, Loewenstein A, Larsen M, Souied E, Schlingemann R, Eldem B, Mones J, Richard G, Bandello F, European Society of Retina Specialists (2014) Guidelines for the management of neovascular age-related macular degeneration by the European Society of Retina Specialists (EURETINA). Br J Ophthalmol 98(9):1144–1167. doi:10.1136/bjophthalmol-2014-305702

Schmier JK, Covert DW, Lau EC (2012) Patterns and costs associated with progression of age-related macular degeneration. Am J Ophthalmol 154(4):675–681. doi:10.1016/j.ajo.2012.04.017, e671

Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, Mickunas E, Gay R, Klimanskaya I, Lanza R (2012) Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 379(9817):713–720. doi:10.1016/S0140-6736(12)60028-2

Schwartz SD, Regillo CD, Lam BL, Eliott D, Rosenfeld PJ, Gregori NZ, Hubschman JP, Davis JL, Heilwell G, Spirn M, Maguire J, Gay R, Bateman J, Ostrick RM, Morris D, Vincent M, Anglade E, Del Priore LV, Lanza R (2015) Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. Lancet 385(9967):509–516. doi:10.1016/S0140-6736(14)61376-3

Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT et al (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA 272(18):1413–1420

Sieving PA, Caruso RC, Tao W, Coleman HR, Thompson DJ, Fullmer KR, Bush RA (2006) Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF

delivered by encapsulated cell intraocular implants. Proc Natl Acad Sci U S A 103(10): 3896–3901. doi:10.1073/pnas.0600236103

Smith W, Assink J, Klein R, Mitchell P, Klaver CC, Klein BE, Hofman A, Jensen S, Wang JJ, de Jong PT (2001) Risk factors for age-related macular degeneration: pooled findings from three continents. Ophthalmology 108(4):697–704

Suter M, Reme C, Grimm C, Wenzel A, Jaattela M, Esser P, Kociok N, Leist M, Richter C (2000) Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. J Biol Chem 275(50):39625–39630. doi:10.1074/jbc.M007049200

Tao W, Wen R, Goddard MB, Sherman SD, O'Rourke PJ, Stabila PF, Bell WJ, Dean BJ, Kauper KA, Budz VA, Tsiaras WG, Acland GM, Pearce-Kelling S, Laties AM, Aguirre GD (2002) Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. Investig Ophthalmol Vis Sci 43(10):3292–3298

Tarallo V, Hirano Y, Gelfand BD, Dridi S, Kerur N, Kim Y, Cho WG, Kaneko H, Fowler BJ, Bogdanovich S, Albuquerque RJ, Hauswirth WW, Chiodo VA, Kugel JF, Goodrich JA, Ponicsan SL, Chaudhuri G, Murphy MP, Dunaief JL, Ambati BK, Ogura Y, Yoo JW, Lee DK, Provost P, Hinton DR, Nunez G, Baffi JZ, Kleinman ME, Ambati J (2012) DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. Cell 149(4):847–859. doi:10.1016/j.cell.2012.03.036

Thanos CG, Bell WJ, O'Rourke P, Kauper K, Sherman S, Stabila P, Tao W (2004) Sustained secretion of ciliary neurotrophic factor to the vitreous, using the encapsulated cell therapy-based NT-501 intraocular device. Tissue Eng 10(11-12):1617–1622. doi:10.1089/ten.2004.10.1617

Tseng WA, Thein T, Kinnunen K, Lashkari K, Gregory MS, D'Amore PA, Ksander BR (2013) NLRP3 inflammasome activation in retinal pigment epithelial cells by lysosomal destabilization: implications for age-related macular degeneration. Investig Ophthalmol Vis Sci 54(1):110–120. doi:10.1167/iovs.12-10655

Velilla S, Garcia-Medina JJ, Garcia-Layana A, Dolz-Marco R, Pons-Vazquez S, Pinazo-Duran MD, Gomez-Ulla F, Arevalo JF, Diaz-Llopis M, Gallego-Pinazo R (2013) Smoking and age-related macular degeneration: review and update. J Ophthalmol 2013:895147. doi:10.1155/2013/895147

Vingerling JR, Dielemans I, Hofman A, Grobbee DE, Hijmering M, Kramer CF, de Jong PT (1995) The prevalence of age-related maculopathy in the Rotterdam Study. Ophthalmology 102(2):205–210

Wang Y, Hanus JW, Abu-Asab MS, Shen D, Ogilvy A, Ou J, Chu XK, Shi G, Li W, Wang S, Chan CC (2016) NLRP3 upregulation in retinal pigment epithelium in age-related macular degeneration. Int J Mol Sci 17(1):73. doi:10.3390/ijms17010073

Wilkinson JT, Fraunfelder FW (2011) Use of herbal medicines and nutritional supplements in ocular disorders: an evidence-based review. Drugs 71(18):2421–2434. doi:10.2165/11596840-000000000-00000

Wong RW, Richa DC, Hahn P, Green WR, Dunaief JL (2007) Iron toxicity as a potential factor in AMD. Retina 27(8):997–1003. doi:10.1097/IAE.0b013e318074c290

Wong TY, Chakravarthy U, Klein R, Mitchell P, Zlateva G, Buggage R, Fahrbach K, Probst C, Sledge I (2008) The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology 115(1): 116–126. doi:10.1016/j.ophtha.2007.03.008

Wong WT, Kam W, Cunningham D, Harrington M, Hammel K, Meyerle CB, Cukras C, Chew EY, Sadda SR, Ferris FL (2010) Treatment of geographic atrophy by the topical administration of OT-551: results of a phase II clinical trial. Investig Ophthalmol Vis Sci 51(12):6131–6139. doi:10.1167/iovs.10-5637

Wong WT, Dresner S, Forooghian F, Glaser T, Doss L, Zhou M, Cunningham D, Shimel K, Harrington M, Hammel K, Cukras CA, Ferris FL, Chew EY (2013) Treatment of geographic

atrophy with subconjunctival sirolimus: results of a phase I/II clinical trial. Investig Ophthalmol Vis Sci 54(4):2941–2950. doi:10.1167/iovs.13-11650

Woodell A, Rohrer B (2014) A mechanistic review of cigarette smoke and age-related macular degeneration. Adv Exp Med Biol 801:301–307. doi:10.1007/978-1-4614-3209-8_38

Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, Gregori G, Penha FM, Moshfeghi AA, Zhang K, Sadda S, Feuer W, Rosenfeld PJ (2014) Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. Ophthalmology 121(3):693–701. doi:10.1016/j.ophtha.2013.09.044

Zeng S, Whitmore SS, Sohn EH, Riker MJ, Wiley LA, Scheetz TE, Stone EM, Tucker BA, Mullins RF (2016) Molecular response of chorioretinal endothelial cells to complement injury: implications for macular degeneration. J Pathol 238(3):446–456. doi:10.1002/path.4669

Zhang K, Hopkins JJ, Heier JS, Birch DG, Halperin LS, Albini TA, Brown DM, Jaffe GJ, Tao W, Williams GA (2011) Ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for treatment of geographic atrophy in age-related macular degeneration. Proc Natl Acad Sci U S A 108(15):6241–6245. doi:10.1073/pnas.1018987108

Zhang J, Kiser PD, Badiee M, Palczewska G, Dong Z, Golczak M, Tochtrop GP, Palczewski K (2015) Molecular pharmacodynamics of emixustat in protection against retinal degeneration. J Clin Invest 125(7):2781–2794. doi:10.1172/JCI80950

Zhang S, Yu N, Zhang R, Zhang S, Wu J (2016) Interleukin-17A induces IL-1beta secretion from RPE cells via the NLRP3 inflammasome. Investig Ophthalmol Vis Sci 57(2):312–319. doi:10.1167/iovs.15-17578

Zhao C, Yasumura D, Li X, Matthes M, Lloyd M, Nielsen G, Ahern K, Snyder M, Bok D, Dunaief JL, LaVail MM, Vollrath D (2011) mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoreceptor degeneration in mice. J Clin Invest 121(1):369–383. doi:10.1172/JCI44303

Hereditary Retinal Dystrophy

Thomas C. Hohman

Contents

Abstract

As our understanding of the genetic basis for inherited retinal disease has expanded, gene therapy has advanced into clinical development. When the gene mutations associated with inherited retinal dystrophies were identified, it became possible to create animal models in which individual gene were altered to match the human mutations. The retina of these animals were then characterized to assess whether the mutated genes produced retinal phenotypes characteristic of disease-affected patients. Following the identification of a subpopulation of patients with the affected gene and the development of techniques for the viral gene transduction of retinal cells, it has become possible to deliver a copy of the normal gene into the retinal sites of the mutated genes. When this was performed in animal models of monogenic diseases, at an early stage of retinal degeneration when the affected cells remained viable, successful gene augmentation corrected the structural and

T.C. Hohman (✉)
Envision Consulting LLC, Ocean City, NJ, USA
e-mail: ThomasCHohman@gmail.com

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_91

functional lesions characteristic of the specific diseases in the areas of the retina that were successfully transduced. These studies provided the essential proof-of-concept needed to advance monogenic gene therapies into clinic development; these therapies include treatments for: *Leber's congenital amaurosis type 2*, caused by mutations to *RPE65*, retinoid isomerohydrolase; *choroideremia*, caused by mutations to *REP1*, Rab escort protein 1; *autosomal recessive Stargardt disease*, caused by mutations to *ABCA4*, the photoreceptor-specific ATP-binding transporter; *Usher 1B disease* caused by mutations to *MYO7A*, myosin heavy chain 7; *X-linked juvenile retinoschisis* caused by mutations to *RS1*, retinoschisin; *autosomal recessive retinitis pigmentosa* caused by mutations to *MERTK*, the proto-oncogene tyrosine-protein kinase MER; *Leber's hereditary optic neuropathy* caused by mutations to *ND4*, mitochondrial nicotinamide adenine dinucleotide ubiquinone oxidoreductase (complex I) subunit 4 and *achromatopsia*, caused by mutations to *CNGA3*, cyclic nucleotide-gated channel alpha 3 and *CNGB3*, cyclic nucleotide-gated channel beta 3. This review includes a tabulated summary of treatments for these monogenic retinal dystrophies that have entered into clinical development, as well as a brief summary of the preclinical data that supported their advancement into clinical development.

Keywords
ABCA4 • Achromatopsia • Adeno-associated viral vectors • Choroideremia • CNGA3 • CNGB3 • Gene augmentation • Gene therapies • Inherited retinal degenerative dystrophies • Leber's congenital amaurosis • Leber's hereditary optic neuropathy • Lentiviral vector • MERTK • MYO7 • ND1 • ND4 • ND6 • REP1 • Retinitis pigmentosa • Retinoschisin • RPE65 • RS1 • Stargardt disease • Usher disease • X-linked juvenile retinoschisis

1 Introduction

The inherited orphan retinal degenerative dystrophies (IRDs) are a group of diseases that lead to the loss of functional vision and often progressing to blindness. They affect ~200,000 people in the USA and ~4.5 million worldwide. IDR include both non-syndromic diseases such as retinitis pigmentosa (RP) and Stargardt disease (STGD), as well as syndromic diseases such as Usher disease that affects hearing and vision, and Bardet–Biedl syndrome that affects vision as well as many other metabolic and physical characteristics. Since the first genetic cause of a form of RP was identified by Dryja et al. (1990), significant progress has been made in the identification of the genetic basis of the other IRDs.

Animal models have been the key to the development of gene therapies for IRDs. There are relatively few naturally occurring animal models for IRDs. Most occur in mice (Hafezi et al. 2000), although the RCS rat with a mutation in the MRTK gene is widely studied. There are no nonhuman primate models, but naturally occurring models have been identified in dogs, cats, and to a more restricted extent in chicken,

sheep, and horses. The identification of pathogenic genes and mutations in humans allowed the creation of models in rodents and larger animals using knockout, overexpression, and more recently gene editing technologies. While the creation of these new models has opened up IRD to therapeutic development, these models have some limitations and not all of the diseases can be modeled. For example, STGD, a juvenile onset macular dystrophy typically caused by mutation of the ABCA4 gene, is poorly modeled by ABCA4 mutations in the mouse strain C57BL/6 (Weng et al. 1999). While these abcr$^{-/-}$ mice model some of the pathologic characteristics of the human disease, such as accumulation of lipofuscin, they fail to develop significant loss of visual function. Whether this reflects a strain issue or a more fundamental challenge remains to be clarified. Similarly, mouse models of many of the Usher syndrome (Ush) mutations often develop hearing but not visual function loss. The absence of a calyceal structure in the photoreceptor outer segments has been suggested to be responsible in part for this lack of phenotype, but this also remains to be further clarified. If this is true, it further limits the development of suitable Usher models since in addition to nonhuman primates, substantial calyceal structures are found in only a few model systems such as pigs and bullfrogs. Rodents also lack a cone-rich, high visual acuity region in the retina equivalent to the fovea centralis in humans, which can also limit the utility of the rodent models.

With the knowledge gained from studies of the types of models describe above, it is now possible to segregate inherited retinal diseases into two broad categories, simple (mono-, di-, and tri-genetic) and multifactorial. Gene augmentation therapies for the treatment of monogenic disease are rapidly advancing. While these gene therapies have the potential for curing the monogenic retinal dystrophies, the benefits of these treatments will likely be limited by the stage of the disease at which viral transduction is performed, as well as the ability of the current retrovirus vectors to transduce large areas of the human retina and to target specific cell types within the retina. Multifactorial diseases remain a major challenge to drug development; nevertheless, having models with similar gene mutations but retinal lesions that progress at different rates, such as the rd1 (Pde6b^{rd1}) and rd10 (Pde6b^{rd10}) mouse models (Farber and Lolley 1976; Chang et al. 2002), and having rodent and non-rodent species with similar gene mutation, such as the P23H rat and pig models (Ross et al. 2012; Orhan et al. 2015), creates the possibility of screening agents that, while not curing the disease, have the potential to delay the progression to blindness.

For the majority of IRD patients, there are no FDA- or EMA-approved therapies. There is one FDA-approved device in the USA, the Argus II prosthetic device. This device, consisting of a camera mounted on a pair of eye glasses, a control unit, and an epiretinal implant containing 55 electrodes, provides the wearer with a low resolution image. It is currently available for use in patients with late stage retinitis pigmentosa, choroideremia (CHM), and atrophic AMD. In addition to the Argus II, in Europe the IRIS II, a second prosthetic device with 150 electrodes, is also approved for people with vision loss from outer retinal degeneration. In addition to these devices, there is a nutritional therapy consisting of a combined treatment regimen of vitamin A palmitate (15,000 IU per day), oily fish (one to two 3-ounce servings of salmon, tuna, mackerel, herring, or sardines per week or 200 mg of docosahexaenoic acid per day), and lutein (12 mg per day), that is recommended by

a number of retinal specialists to slow vision loss in people with retinitis pigmentosa and Usher syndrome types 2 and 3. This recommendation is based on the pioneering clinical research of Berson who produced an actuarial chart to predict the benefit of this dietary therapy in prolonging functional vision (Berson 2007). Concerns, however, have been expressed that this supplement could exacerbate some forms of RP such as those due to mutations in ABCA4.

There are many clinical trials investigating interventions for IRDs based on small molecules, proteins, nutritionals, and devices, as well as gene and stem cell therapies. Table 1 lists treatments that have entered into clinical development and are listed in ClinTrials.gov, a searchable registry and database of federally and privately supported clinical trials conducted in the USA and around the world. Similar databases such as the European Clinical Trials Register are kept by other nations. A number of excellent reviews of therapies in development for the treatment of IRDs have been published recently (Pearson et al. 2014; Tucker et al. 2014; Sowden 2015; Sahel et al. 2015; Thompson et al. 2015; Veleri et al. 2015). Gene augmentation therapies for monogenic inherited retinal diseases are the major focus of this review.

Table 1 Treatments for inherited retinal dystrophies that have entered into clinical development and are listed in ClinTrials.gov

Disease indication	Sponsor	Compound/treatment	ClinTrial.gov study identification number
Leber congenital amaurosis (RPE65 mutations)	Spark Therapeutics	RPE65 gene replacement therapy SPK-RPE65 AAV2-hRPE65v2	NCT00516477 NCT00999609 NCT01208389
	Applied Genetic Technologies Corp	RPE65 gene replacement therapy AAV-RPE65 rAAV2-CB-hRPE65	NCT00749957
	Hadassah Medical Organization	RPE65 gene replacement therapy rAAV2-hRPE65	NCT00821340
	University College, London	RPE65 gene replacement therapy rAAV2-hRPE65AAV2/5 OPTIRPE65	NCT02781480
	University of Pennsylvania National Eye Institute	RPE65 gene replacement therapy rAAV2-CBSB-hRPE65	NCT00481546
Leber hereditary optic neuropathy	University of Miami National Eye Institute	ND4 gene replacement therapy scAAV2-P1ND4v2	NCT02161380
	GenSight Biologics	ND4 gene replacement therapy rAAV2/2-ND4 GS010	NCT02064569 NCT02652767 NCT02652780
	Stealth BioTherapeutics Inc	Elamipretide (MTP-131)	NCT02693119
	Santhera Pharmaceuticals	Idebenone	NCT02774005
	Raptor Pharmaceuticals Inc	RP103 Cysteamine bitartrate delayed-release capsules	NCT02473445 NCT02023866

(continued)

Table 1 (continued)

Disease indication	Sponsor	Compound/treatment	ClinTrial.gov study identification number
Stargardt macular degeneration	Sanofi	ABCA4 gene replacement therapy EIAV-CMV-GFP SAR422459	NCT01367444 NCT01736592
	Astellas Institute for Regenerative Medicine (Ocata)	Human embryonic stem cell derived retinal pigmented epithelial MA09-hRPE	NCT02445612
	Southwest Hospital, China	Human embryo stem cell derived retinal pigment epithelium	NCT02749734
	CHA Biotech Co., Ltd	Embryonic stem cell derived retinal pigmented epithelial cells (MA09-hRPE)	NCT01625559
	Retina Associates of South Florida	Autologous bone marrow-derived stem cells (BMSC)	NCT01920867
	Alkeus	ALK-001 C20-D3-retinyl acetate	NCT02230228 NCT02402660
	Catholic University of the Sacred Heart	Saffron supplementation	NCT01278277
	University of Utah	Docosahexaenoic Acid (DHA) dietary supplement	NCT00420602
Usher syndrome	Sanofi	Myosin VIIa gene replacement therapy UshStat EIAV-CMV-MYO7A	NCT01505062 NCT02065011
	Neurotech Pharmaceuticals	Encapsulated Ciliary Neurotrophic Factor (CNTF)	NCT01530659
Choroideremia	Spark Therapeutics	REP-1 gene replacement therapy AAV2-hCHM	NCT02341807
	NightstaRx	REP-1 gene replacement therapy rAAV2.REP1	NCT02077361 NCT02553135 NCT01461213
	STZ eyetrial	REP-1 gene replacement therapy rAAV2.REP1	NCT02671539 NCT01461213
	Pixium Vision SA	Intelligent retinal implant system	NCT02670980 NCT01864486
X-linked retinoschisis	Applied Genetic Technologies Corp	RS-1 gene replacement therapy rAAV2tYF-CB-hRS1	NCT02416622
	National Eye Institute	RS-1 gene replacement therapy AAV-RS1	NCT02317887

(continued)

Table 1 (continued)

Disease indication	Sponsor	Compound/treatment	ClinTrial.gov study identification number
Retinitis pigmentosa	Sheba Medical Center	*Dunaliella bardawil* (9-cis β carotene)	NCT01680510
	Retro-Sense Therapeutics	RST-001	NCT02556736
	Chaitanya Hospital, Pune	Autologous bone marrow-derived mononuclear stem cell	NCT01914913
	ReNeuron	Human retinal progenitor cells	NCT02464436
	jCyte	jCell (human allogeneic retinal progenitor cells)	NCT02320812
	Mahidol University	Bone marrow-derived mesenchymal stem cells	NCT01531348
	Red de Terapia Celular	Autologous bone marrow stem cell	NCT02280135
	Stem Cells Arabia	Autologous bone marrow-derived CD34+, CD133+, and CD271+ stem cell	NCT02709876
	University of California, Davis	Autologous intravitreal bone marrow CD34+ stem cells	NCT01736059
	Retina Associates of South Florida	Autologous bone marrow-derived stem cells	NCT01920867
	Bayer	Aflibercept	NCT02661711
	The Hong Kong Polytechnic University	*Lycium barbarum*	NCT02244996
	Beirut Eye Specialist Hospital	L-Dopa	NCT02837640
	Neurotech Pharmaceuticals	Encapsulated Ciliary Neurotrophic Factor (CNTF)	NCT01530659
	National Eye Institute	Minocycline	NCT02140164
	King Faisal Specialist Hospital and Research Center	MERTK gene replacement therapy rAAV2-VMD2-hMERTK	NCT01482195
	Ospedale San Raffaele	Recombinant human nerve growth factor (rhNGF)	NCT02609165
	Dompé Farmaceutici S.p.A	Recombinant human nerve growth factor (rhNGF)	NCT02110225
	National Eye Institute	Interferon gamma-1b	NCT02338973
	Pixium Vision SA	Intelligent retinal implant system	NCT02670980 NCT01864486
	Retina Implant AG	Retina implant model alpha	NCT01024803 NCT02720640 NCT02588430
	Second Sight Medical Products	Argus II retinal stimulation system	NCT00407602 NCT01999049 NCT02303288 NCT01860092 NCT01490827

(continued)

Table 1 (continued)

Disease indication	Sponsor	Compound/treatment	ClinTrial.gov study identification number
Achromatopsia	STZ eyetrial	CNGA3 gene replacement therapy rAAV.hCNGA3	NCT02610582
	Applied Genetic Technologies Corp	CNGA3 gene replacement therapy rAAV2tYF-PR1.7-hCNGB3	NCT02599922

2 Monogenic Diseases

Monogenetic diseases are attractive targets for genetic therapy. Corrections of the genetic mutation, or genetic modifications that remove the deleterious activity of the mutant gene product, have tremendous therapeutic potential. While the naming of the IRDs, such as retinitis pigmentosa (RP), Usher syndrome (Ush), Leber's congenital amaurosis (LCA), and Bardet–Biedl syndrome, implies a small group of well-defined diseases, it hides a genetic complexity in which many different genes are implicated. For instance, the three forms of RP (autosomal dominant, autosomal recessive, and X-linked) are caused by mutations in at least 58 different genes, and these known mutations account for only ~75% of diagnosed cases. Genes for the remaining 25% have not been identified. As a group, IRDs are due to mutations in over 280 different genes. Within each implicated gene, many different mutations have been identified in the regulatory, coding, and intervening sequences, adding to the genetic diversity. Furthermore, different mutations within the same gene may underlie different IRDs. For example, mutations to RPE65 (enzyme responsible for photoisomerization of 11-cis retinal to all-trans retinal, initiating the phototransduction pathway) are associated with LCA and RP, while mutations to CRX (photoreceptor-specific transcription factor) are associated with RP, LCA, and cone-rod dystrophies. This complexity created the current trend to identify disease targets, not for named diseases, but rather for gene-based designation such as RPE65 disease and CEP290 (290 kDa centrosomal protein) disease.

The most advanced IRD project – gene augmentation for LCA type 2, in which a mutant RPE65 gene is supplemented by adeno-associated virus delivery of wild-type copies of the gene (voretigene neparvovec) – is very promising, but may represent the low hanging fruit, and not be illustrative of IRDs as a whole. Clinically relevant therapies must take into consideration the relevant window of intervention, i.e., administration prior to the degeneration of the target cells. While many preclinical studies seek to treat animals soon after birth, this is not yet possible in human clinical studies. In RPE65 disease, retinal pigmented epithelial (RPE) cells affected by the gene mutation are mature, viable cells that lack RPE65 enzyme activity. Restoration of this activity revives the visual cycle. Developing

gene therapies for other IRDs may be considerably more challenging. Gene augmentation without regulated expression may not be sufficient for correcting the mutation of genes that encode developmentally regulated proteins such as harmonin, an anchoring/scaffolding protein in cochlear hair cells (Ush type 1C), or correcting mutations to structural components such as CEP 290, a centrosomal protein involved in ciliary assembly (LCA type 10). Autosomal dominant diseases also require different strategies depending on whether the mutant activity is a simple haplo-insufficiency such as X-linked RP or a dominant negative activity with potential toxicity such as P23H, a single base-substitution at the codon position 23 in the human opsin gene in RP. To address this genetic complexity, many different strategies will be required and some of these have not yet advanced beyond the concept stage.

The most clinically advanced IRD genetic therapies utilize a gene augmentation strategy for recessive diseases in which a wild-type cDNA sequence of the mutated gene, in a retroviral expression cassette, is delivered to the retina. There are at least nine different gene augmentation therapies currently in clinic trials: (1) retinoid isomerohydrolase, for LCA type 2 and RP type 20; (2) REP1, Rab escort protein 1, for choroideremia (CHM), (3) ABCA4, the photoreceptor-specific ATP-binding transporter, for STGD; (4) MYO7A, myosin heavy chain 7, for Ush type Ib; (5) CNGA3, cyclic nucleotide-gated channel alpha 3, for achromatopsia); (6) CNGB3, cyclic nucleotide-gated channel beta 3, or achromatopsia; (7) MERTK, the proto-oncogene tyrosine-protein kinase MER, for RP type 28; (8) ND4, mitochondrial nicotinamide adenine dinucleotide ubiquinone oxidoreductase (complex I) subunit 4, for *Leber's hereditary optic neuropathy* (LHON); and (9) RS1, retinoschisin, for X-linked juvenile retinoschisis (XLRS). The preferred vectors have been adeno-associated virus with the (AAV) serotypes, although therapies for ABCA4 and MYO7A disease use viral constructs created with the equine infectious anemia lentivirus (EIAV) vectors. Most of the therapies listed above are delivered to the RPE or photoreceptors via a subretinal injection, but intravitreal injections are being explored in XLRS gene therapy due to the fragile nature of the retina in this disease. Different viral serotypes when administered via subretinal or intravitreal injections can target different cells; different gene expression promoters can add further cell type specificity to the constructs. While it is tempting to think of a given gene-targeted therapy (e.g., RPE65) delivered by a specific serotype (e.g., AAV2) as a single drug, differences in the constructs, their control elements, manufacturing, formulation, and their surgical delivery into the retina yield very different results. It is therefore important to consider each gene construct as a unique drug with unique characteristics and efficacy.

2.1 RPE65 Gene Therapy

The RPE65 gene encodes retinoid isomerohydrolase, a 65 kDa protein with 533 amino-acid residues, that is expressed almost exclusively in RPE cells. The role for this enzyme is to maintain the visual cycle by regenerating 11-cis retinol

following its photoisomerization to all-trans retinal. After its formation in photoreceptor outer segments, all-trans retinal is reduced to all-trans retinol and transported to RPE cells where it is esterified by lecithin retinol acyltransferase, converted to 11-cis retinol by RPE65, and then transported back to rod outer segments where it is oxidized to 11-cis retinal and conjugated with opsin to form rhodopsin. The absence of RPE65 activity is accompanied by a marked reduction in 11-cis retinal levels and an accumulation of retinal esters in RPE cells (Travis et al. 2007). The loss of RPE65 activity appears to affect cone photoreceptors, as evidenced in experiments in which mice lacking the NRL transcription factor ($Nrl^{-/-}$) and forming only cone photoreceptors were crossed with $RPE65^{-/-}$ mice. At 4 weeks of age, mice homozygous for both null mutations formed approximately 9 rows of photoreceptor nuclei in the retinal outer nuclear layer; by 12 weeks of age, the number of rows of nuclei decreased by about 50%. These ultrastructural changes were accompanied by the loss of ERG responses typical of cone photoreceptors (Feathers et al. 2008). In $Rpe65^{-/-}$ mice, the accumulation of retinal esters in RPE cells is accompanied by a 50% decrease in photoreceptors between 1 and 18 months of age. Experiments performed by Rohrer and colleagues (2003) have demonstrated that the surviving rod photoreceptors remain functional. Injections of 11-cis retinal resulted in the regeneration of visual pigment and an improvement of scotopic ERG a- and b-waves. These observations support the prospect for gene augmentation therapy. The role of RPE65 and the potential for gene therapy have been recently reviewed (Cai et al. 2009).

Mutations to RPE65 cause LCA type 2 and autosomal recessive retinitis pigmentosa 20 (RP20). LCA is an autosomal recessive form of retinal degeneration characterized by nystagmus, decreased pupillary responses, and severe vision loss. A more complete description of LCA is available from Weleber et al. (2013). LCA is one of the most common causes of inherited blindness in children with a prevalence of 2–3 per 100,000 individuals. There are 18 types of LCA, each characterized by specific gene mutations; type 2 LCA, characterized by mutations to RPE65, is found in approximately 3–16% of individuals with LCA (Chacon-Camacho and Zenteno 2015). A wide range of disease severity from congenital blindness to adult-onset retinitis pigmentose has been associated with RPE65 mutations; the most common phenotype is severe and early onset retinal degeneration (Thompson et al. 2000).

Gene therapy for RPE65 mutations is the most clinically advanced therapy; it has been developed independently by at least 6 groups around the world. All but one group selected the same viral serotype, AAV2 which has the ability to infect nondividing cells and to stably integrate into the host cell genome at a specific site. Similarly, most groups used the chicken β-actin (CBA) promoter, although an RPE65 gene construct with the native human RPE65 promoter is also in the clinic. Delivery is via a subretinal injection, although the site of injection relative to the macular differs between products, as does the injection protocol.

Efficacy of RPE65 gene therapy was first demonstrated (Acland et al. 2001) for a naturally occurring mutation identified in a Briard dog that produced a truncated RPE65 protein (Aguirre et al. 1998; Vesk et al. 1999). Treatment of a single eye led to improvements in pupillary responses, nystagmus, electroretinography (ERG),

and visual behavior that was not mirrored in the untreated eye. Subsequently, efficacy, as measured by improvements in ERG, was demonstrated in both a mouse RPE-65 knockout model (Redmond et al. 1998; Bennicelli et al. 2008) and a naturally occurring RPE65-deficient mouse model, rd12 (Pang et al. 2005; Bennicelli et al. 2008). Long-term follow-up in studies of Briard dogs provided the proof-of-concept needed to advance RPE65 gene therapy into clinical studies (Acland et al. 2005; Narfstrom et al. 2005; Le Meur et al. 2007). While one group entered into clinical studies based only on mouse and rodent studies (MaGuire 2005), a second group included toxicology testing in normal cynomolgus monkeys (Jacobson et al. 2006) before entering clinical trials (Jacobson et al. 2012).

For safety reasons, initial preclinical and clinical studies addressed unilateral eye injections, but the long-term goal is bilateral correction. Intravitreal injections in mice, performed on each eye, 1 month apart, have elicited an immune response against the AAV capsid proteins, leading to very poor gene expression in the second eye, compared to the first eye (Li, Miller et al. 2008, 2009). In contrast, subretinal injections in both mice (Li et al. 2009) and dogs (Acland et al. 2005) have achieved similar levels of expression in each eye, even when the second eye was treated 85–180 days after the first; these results may reflect a difference in immune privilege between the vitreous cavity and subretinal space. The safety of repeated subretinal injections of AAV capsid proteins has also been observed in clinical trials in which vector administration to the second eye occurred up to 3.3 years after the first eye was treated (Bennett et al. 2012).

Clinical outcomes for the different RPE65 drugs vary. Although, not yet published in a peer-reviewed journal, Spark Therapeutics (www.sparktx.com) has described the results of a phase 3 clinical trial of their product, voretigene neparvovec (NCT00999609), that enrolled 31 patients, with 21 receiving active treatment. The study met its primary endpoint, demonstrating an improvement in bilateral mobility testing between baseline and 1 year ($p = 0.001$). Secondary endpoints of improvements in full-field light sensitivity threshold testing and mobility test change score for the first injected eye showed statistical significance ($p = 0.001$), but no improvement in vision was observed. No serious adverse events or immune responses were reported. In a separate clinical study (Cideciyan et al. 2013), gene therapy improved vision for at least 3 years, but photoreceptor degeneration continued; similar observations have been reported in the Briard dog model when therapy was administered at a late stage in the disease process, equivalent to the disease stage of patients in this clinical trial (Cideciyan et al. 2013). Despite the decline in the retinal sensitivity of the transduced area that was observed in the Cideciyan study, visual function remained greater than that in the surrounding areas that were not transduced (Jacobson et al. 2015). In a phase 1–2 open label study of two doses of RPE65 gene therapy in 12 patients, Bainbridge and collaborators reported improvements in retinal sensitivity in 6 of the study participants; this improvement peaked at 6–12 months after treatment, and then declined (Bainbridge et al. 2015). These improvements in retinal sensitivity were not accompanied by improvements in ERG, nor consistent improvements in visual acuity. Intraocular inflammation or immune responses were observed in five of the eight patients

receiving the higher dose, but in none of the patients receiving the lower dose, raising concern about dose-limiting toxicity. Notably this group not only used a different vector construct but also used a different injection protocol; in this protocol the gene transcript was administered in a volume that was 2 times greater than that used by others, 0.9–1 ml, and injection of this larger volume formed a subretinal bleb that extended into the fovea. An understanding of the durability of gene therapy awaits longitudinal studies by other groups to determine if the decline in retinal sensitivity is a drug-specific finding, an injection protocol-specific finding, or a more general outcome.

2.2 MYO7A Gene Therapy

The MYO7A gene encodes myosin VIIA, an unconventional myosin with a very short tail; this protein is expressed in the inner ear and retina. In the inner ear, myosin VIIA plays a role in the development and maintenance of stereocilia, critical for converting sound waves to nerve impulses. Stereocilia are also elements of the vestibular system, responsible for maintaining balance. Within the retina, myosin 7A is localized within RPE cells and is responsible for the movement of melanosomes. Mutation to the MYO7A gene has been linked to Ush type IB.

Ush is characterized as a congenital, bilateral, profound sensorineural hearing loss with impaired bilateral vestibular function and adolescent-onset retinitis pigmentosa. Earlier prevalence estimates reported that Ush affected 4–5 in 100,000 individuals. However, these estimates probably under represented less severely affected populations. More recent estimates suggest that the prevalence may be as high as 1 in 6000 individuals (Kimberling et al. 2010). Ush is typically separated into three clinical types based on the severity and onset of hearing and vision loss; the most severely affected and the most common are type 1 individuals, who are born deaf and begin to lose vision in their first decade. In one study, Ush type 1 represented about 70% of those with Ush (Tamayo et al. 1991). Ush type 1 can be caused by mutations in any one of several different genes that function in the development of stereocilia. For this reason, type 1 disease is further segregated by specific gene mutations, these include type 1B with mutations to MYO7A, type 1C with mutations to USH1C (harmonin), type 1D with mutations to CHD23 (cadherin 23), type 1F with mutations to PCDH15 (protocadherin), and type 1G with mutations to USH1G (Sans, part of a protein complex linking cadherin to stereocilia microfilaments). Type 1B accounts for 50–60% of all patients with Ush type 1 (Lentz and Keats 2016).

Efforts to developing gene therapy for Ush1B disease (MYO7A) have experienced challenges different from those for RPE65 mutations. The greatest challenge is deciding whether to proceed into clinical studies in the absence of strong preclinical data. The most common Ush1B model, the shaker1 ($sh1^{-/-}$; $shaker^{4626SB/4626SB}$) mouse, does not develop some of the diagnostic characteristics found in patients with Ush1B disease. $sh1^{-/-}$ mice are homozygous for a nonsense mutation in the MYO7A gene and exhibit a distinct phenotype that includes cochlear and vestibular

dysfunction, slower transport of rhodopsin leading to its accumulation at the photoreceptor connecting cilium and slower distal migration of photoreceptor outer segments (Liu et al. 1999), an elevated threshold of light for translocation of α-transducin from the outer to the inner segments of photoreceptors (Peng et al. 2011), mis-localization of melanosomes (Liu et al. 1998), and slower phagosome motility resulting in slower outer segment digestion (Gibbs et al. 2003). However, unlike Ush1B patients, sh1$^{-/-}$ mice do not exhibit an ERG decline, nor photoreceptor degeneration (Gibson et al. 1995; Libby and Steel 2001) although mild changes have been reported.

An additional challenge for MYO7A gene therapy is the size of the protein, 2215 amino-acid residues; the size of the cDNA for this protein exceeds the packaging limits of AAV. Early proof-of-concept studies used HIV-1 derived lentiviral vectors and a constitutive CMV promoter to demonstrate that defects in phagosome digestion and melanosome motility could be rescued in primary cultures of RPE cells (Hashimoto et al. 2007). This was supported in vivo, by restoration of the localization of melanosomes in RPE cells, and correction of the abnormal accumulation of opsin (Hashimoto et al. 2007).

The clinical development of MYO7A gene therapy was built upon the use of an EIAV lentiviral vector that restored normal light threshold for α-transducin translocation in the shaker1 mice and protected photoreceptors from acute and chronic light induced cell degeneration (Allocca et al. 2008). Toxicity and biodistribution were assessed in a 3-month study of the SAR421869 construct following a single subretinal injection in rhesus macaques (*Macaca mulatta*) (Zallocchi et al. 2014) prior to entering into clinical trials. The ongoing phase I/II trials (NCT01505062, NCT02065011) are evaluating the safety of this integrating lentiviral vector.

An initial report that the AAV2/5 pseudotype virus could deliver an oversized MYO7A gene into primary cultures of RPE cells (Allocca et al. 2008) proved to be the result of gene fragmentation to sizes that could be packaged within AAV and subsequently recombined when cotransduced into cells. This has led to substantial preclinical research on multiple vector constructs designed to maximize intracellular recombination (Duan et al. 2001; Reich et al. 2003; Ghosh et al. 2008; Li, Sun et al. 2008; Dong et al. 2010; Lai et al. 2010; Wu et al. 2010; Dyka et al. 2014) that could obviate the use of a lentiviral vector. This may be advantageous in that lentiviruses are integrating viruses that may bring a risk of host insertional mutagenesis.

2.3 ABCA4 Gene Therapy

ABCA4 is a member of the ATP-binding cassette transporter gene subfamily; this gene transcribes a large retina-specific protein, 256 kDa with 2273 amino-acid residues, that consists of two transmembrane domains, two glycosylated extracellular domains, and two nucleotide-binding domains. The ABCA4 protein is almost exclusively expressed in outer segment disk edges of rod photoreceptors where it translocates N-retinylidene-phosphatidylethanolamine from the disk lumen to the

cytoplasmic side of the disk membrane where it can then be converted back to retinol. As a result of the phagocytic turnover of photoreceptor outer segments, the loss of ABCA4 activity leads to the accumulation of N-retinylidene-phosphatidyl-ethanolamine in RPE cells; this accumulation leads to the formation of toxic insoluble bisretinoids that appear to contribute to RPE cell death (Kaminski et al. 2006).

The ABCA4 gene was first cloned and characterized as a gene that causes STGD by Allikmets and colleagues (1997). STGD linked to ABCA4 (STGD1) is the most common childhood recessively inherited macular dystrophy, affecting about 1 in 10,000 individuals. STGD1 is characterized by bilateral progressive atrophy of the RPE with the appearance of orange-yellow flecks distributed around the macula; RPE atrophy causes the loss of central vision that typically starts within the first 20 years of life and progresses to blindness. Rare autosomal dominant forms of Stargardt macular dystrophy STGD3 and STGD4 are linked to ELOVL4 (elongation of very long chain fatty acids protein 4) and PROM1 (prominin1), respectively (Yanoff and Duker 2008). More than 400 sequence variations in the ABCA4 gene have been identified (Allikmets et al. 1997; Sun and Nathans 2000; Kaminski et al. 2006; Braun et al. 2013). While there is no clear genotype–phenotype relationship, Stone's group has suggested that the overall pathogenicity of ABCA4 alleles for a patient can be calculated as the sum of the pathogenicity of each risk allele (Schindler et al. 2010). Mutations to the ABCA4 gene have been associated with other recessive retinal diseases, such as cone-rod dystrophies, some forms of retinitis pigmentosa, and age-related macular degeneration (Weng et al. 1999).

Development of gene therapy for ABCA4 gene disease, currently in clinical trial NCT01367444, faced similar preclinical challenges to those experienced during development of the MYO7A gene therapy. The protein encoding cDNA for ABCA4 with 2,273 amino-acid residues was too large to fit into existing AAV vectors, leading to the use of EIAV vectors (Kong et al. 2008). Additionally, while the Abca4$^{-/-}$ mouse model showed an accelerated accumulation of A2E in RPE cells, a delayed rate of rod photoreceptor dark adaptation, a transient accumulation of all-trans retinaldehyde, and a transient reduction in all-trans retinol following a photobleach, significant retinal degeneration did not develop in animals younger than 8–9 months of age (Song et al. 2014; Veleri et al. 2015). While an exacerbated accumulation of A2E was accompanied by photoreceptor dystrophy at an early age in a double mutant Abca4$^{-/-}$ Rdh8$^{-/-}$(Maeda et al. 2008), there is no evidence of RDH8 involvement in STGD and the relevance of this model for ABCA4 disease is not fully accepted in the community. The clinical program was based on preclinical evidence that the EIAV-bRho-ABCR vector could lead to a statistically significant reduction of A2E accumulation in the Abca4$^{-/-}$ mouse and 6-month safety assessments of the viral construct performed in macaques (*M. mulatta*) and Dutch-belted rabbits. In these studies, there were no abnormal clinical or histopathologic findings in either species. Although antibodies to the vector were detected in rabbits, these findings were not associated with long-term toxicity findings (Binley et al. 2013). NCT01367444 is a Phase 1/2 dose escalation safety study of a

subretinal injected viral construct identified as SAR422459. The primary endpoint is an assessment of safety; the secondary endpoint is a delay in retinal degeneration.

2.4 Choroideremia Gene Therapy

The intracellular traffic of proteins and substrates is regulated by small GTP-binding proteins called Rab proteins. The addition of geranyl-geranyl groups, i.e., prenylation, via geranyl-geranyl-transferase to Rab proteins, is essential for Rab protein target-protein recognition. REP-1 facilitates the docking of newly synthesized Rab proteins with geranyl-geranyl-transferase, forms a stable complex with the prenylated Rab protein, and directs it to its target cellular membrane. Both REP-1 and REP-2 have similar functions and are ubiquitously expressed; REP-2 can compensate for the loss of REP-1 in most tissues, but not in the eye. The absence of REP-1 is linked with choroidal, RPE and photoreceptor degeneration in CHM, an X-linked retinal dystrophy with an estimated prevalence of 1–2 per 100,000 individuals (MacDonald et al. 2003; Tolmachova et al. 2006).

CHM appears to be an attractive target for gene therapy. Not only is this a monogenetic disease, but CHM is relatively simple to diagnose, compared with other inherited blinding diseases, greatly facilitating the identification of the target patient population for drug treatment studies (MacDonald et al. 1998); additionally, retinal degeneration develops slowly and does not begin until the second decade of life, providing a relatively large time frame for therapeutic intervention (Freund et al. 2016). While mutations in the CHM gene include full deletions, partial deletions, deletion/insertions, splice site mutations, and nonsense mutations, immunoblot analysis of protein from white blood cells of CHM patients shows that most patients lack the REP-1 gene product (MacDonald et al. 1998). Despite an understanding of the genetic cause of CHM, development of a gene therapy has been delayed by the limited availability of animal models. A knockout model of the disease, for example, is not readily available because deletion of REP-1 was embryonically lethal in males; it was also lethal in females if the mutated allele is present on the maternal X chromosome, but not if it was present on the paternal X chromosome (van den Hurk et al. 1997). Proof-of-concept of gene augmentation therapy for CHM was first described in 2003. Using an AAV construct of REP-1, Anand and colleagues (2003) were able to successfully rescue REP-1 function in lymphoblasts and fibroblasts isolated from CHM individuals. More recently Black and collaborators (2014) tested an AAV8 construct with human CHM in a conditional knockout mouse model, $Chm^{null/WT}$, in which heterozygous null female carriers exhibited slowly progressive degeneration of the photoreceptors and patchy depigmentation of the RPE. Additional proof-of-concept was established by Vasireddy et al. (2013). These investigators demonstrated the ability of an AAV2 viral construct of the human CHM cDNA under control of the CMV-enhancer chicken beta actin promotor to restore the expression of the Rep-1 protein, the prenylation of Rab27a, and the normal translocation of RAB27protein to the nuclear region in lymphoblasts and induced pluripotent stem cells from CHM

patients. These investigators also performed the initial safety assessment of the viral construct in normal-sighted mice; in these animals there were no short-term degenerative changes in photoreceptors resulting from overexpression of Rep-1 and no evidence of an inflammatory response to the viral construct. These studies paved the way for a human clinical trial of gene therapy for CHM.

Observations through the 6-month time point have been described for a cohort of six patients with advanced disease who received a subretinal injection of an AAV REP1 gene construct in study NCT01461213 (MacLaren et al. 2014). In this multicenter study, the mean gain in best corrected visual acuity (BCVA) was 3.8 letters; two patients with advanced disease gained 21 and 11 letters of BCVA, respectively. Change in BCVA were accompanied by an improvement in scotopic microperimetry. At 3.5 years after treatment (Edwards et al. 2016), BCVA in the two patients with advanced disease had improved by 21 and 18 letters from their baseline measurements, while BCVA in their untreated fellow eyes decreased by 18 and 6 letters, respectively. In addition to the MacLaren study, there are four ongoing clinical trials of gene therapy in patients with CHM (NCT02077361, NCT02553135, NCT02671539, and NCT02341807).

2.5 RS1 Gene Therapy

XLRS is an inherited early onset retinal degenerative disease caused by mutations in the RS1 gene which encodes a discoidin domain-containing protein, retinoschisin, secreted as a homo-oligomeric complex (Sauer et al. 1997). This complex binds tightly to the surface of photoreceptors and bipolar cells helping maintain the cellular organization of the retina, as well as the structure of the photoreceptor-bipolar synapse (Molday et al. 2001). XLRS is characterized by an abnormal splitting of the retinal nerve fiber layer and the ganglion cell layer. RS1 is the only gene known to be associated with XLRS; RS1 missense, splice site, frameshift, insertion, and deletion mutations of RS1 all result in the same phenotype with radial streaks forming within the foveal schisis, splitting of inner retinal layers in the peripheral retina, and visual acuity deteriorating during the first and second decades of life (Tantri et al. 2004). Severe complications such as retinal hemorrhage or retinal detachment occur in up to 40% of patients, especially in older individuals. Males who inherit the pathogenic gene variant will develop the disease; female carriers are asymptomatic. Affected males pass the pathogenic variant to all of their daughters and none of their sons. The estimated prevalence is 1–2 per 100,000 individuals (Molday et al. 2012).

Proof-of-concept of gene augmentation therapy was established in a mouse Rs1 knockout model, $Rs1h^{-/Y}$ (Zeng et al. 2004). Histologic examination of retinal sections from 1- to 6-month-old animals demonstrated intralamellar separations, resembling the retinoschisis observed in XLRS patients; this anatomical change was accompanied by diminished dark-adapted b-wave responses across the stimulus intensity range. At 13 weeks of age, an AAV vector that contained the murine Rs1h cDNA, orthologue of the human RS1 protein, driven by the CMV promoter,

was injected into one eye of Rs1h$^{-/Y}$ mice and the contralateral eye was injected with vehicle. ERG measurements of the eyes treated with the AAV vector construct demonstrated a complete reversal of the b-wave functional abnormality observed in the vehicle-treated eyes. However, the AAV vector construct treatment did not reverse the retinal structure damage evident at 13 weeks of age in Rs1h$^{-/Y}$ mice and hence did not reverse the reduced a-wave amplitude. The effects of gene augmentation therapy were further explored in a mouse Rs1 knockout model using a recombinant AAV serotype 5 containing the human RS1 cDNA under the control of the mouse photoreceptor-specific mouse opsin promoter (AAV5-mOPs-RS1) (Janssen et al. 2008). This study examined the consequences of single subretinal injections administered at various stages of disease. Significant ERG functional improvement was observed in treated versus untreated eyes in retinoschisis-1 deficient mice when the treatment was administered 15 days, 1 month, and 2 months after birth. However, no effect on ERG function was observed when treatment was administered in animals 7 months of age, an age exhibiting advanced retinal disease. Sieving and collaborators have achieved similar results using an rAAV2 virus construct containing the mouse Rs1h cDNA under the control of the general CMV promoter (Kjellstrom et al. 2007; Takada et al. 2008). When Rs1h knockout mice were injected at 14 days of age and examined at 14 months of age, there were 4–5 rows of photoreceptor nuclei in the treated eyes compared to a single row of nuclei in the untreated animals; few photoreceptors in the untreated eye had well-formed inner and outer segments, while the inner and outer segments in the treated eye resembled those of wild-type animals, but were about one-half their length. The waveform of full-field scotopic ERGs from the treated eyes at 14 months of age was similar to that of wild-type animals. In the study by Takada et al. (2008), the viral construct was administered at day 14 and full-field scotopic ERG and retinal histology assessments were performed at 8 months of age. Consistent with the finding of Kjellstrom et al. (2007), histological examination demonstrated a greater number of photoreceptors in the treated eye, but incomplete photoreceptor protection. Similarly, eyes injected with the viral construct exhibited a larger b-wave amplitude compared with that in untreated eyes, resulting in smaller, but essentially normal b- and a-wave configurations.

Recognizing the fragile nature of the retina in XLRS, Park et al. (2009) created a construct in which human retinoschisin cDNA under control of the human retinoschisin promotor was packaged in an AAV type 8 capsid and administered as an intravitreal injection. Eleven weeks after this construct was administered to Rs1 knockout mice, the retinal distribution of human retinoschisin was similar to the distribution of retinoschisin in wild-type animals. Off target expression of retinoschisin was not observed and the mice treated with this vector had improved ERG retinal signaling.

Gene augmentation therapy is now being evaluated in patients with XLRS with two separate studies actively recruiting patients. Study NCT02317887, sponsored by the National Eye Institute, National Institutes of Health, is a Phase 1/2 safety, dose escalation study in which the viral construct AAV8-scRS/IRBPhRS is being administered as an intravitreal injection. Approximately 24 patients with a gene

mutation in the RS1 gene will be recruited; enrollment is limited to patients with BCVA worse than 63 letters, equivalent to 20/50Snellen–2 letters. The primary endpoint is safety, the secondary endpoints include changes in visual function and ERG responses. The second study, NCT02416622 sponsored by Applied Genetic Technologies Corp, has a similar design; the viral construct, rAAV2tYF-CB-hRS1, is also being administered as an intravitreal injection.

2.6 MERTK Gene Therapy

The MERTK gene is a member of the MER/AXL/TYRO3 receptor kinase family and encodes a transmembrane protein with two fibronectin type-III domains, two immunoglobulin-like domains, and one tyrosine kinase domain (Graham et al. 1994). MERTK signaling plays a role in macrophage clearance of apoptotic cells, platelet aggregation, cytoskeleton reorganization, and phagocytic engulfment (Tang et al. 2015). Mutations to the human orthologue of the MERTK gene were first described by Gal et al. (2000); further population studies of individuals with autosomal recessive RP (Petrs-Silva and Linden 2014) suggest that MERTK mutations are a rare cause of retinal dystrophy, affecting fewer than 3% of individuals with autosomal recessive RP (Charbel et al. 2009; Mackay et al. 2010; Tada et al. 2006; Abu-Safieh et al. 2013; Patel et al. 2015).

RP is the most common form of inherited retinal degeneration with a prevalence of 1 in 4,000 individuals. Patients with RP experience night blindness, constriction of peripheral visual field in the early stages, and complete vision loss at later stages. Disease presentation can be limited to the eye or can appear as a syndrome, such as Ush. Non-syndromic RP has been linked to more than 58 genes; most of these genes are expressed in photoreceptors or retinal pigment epithelium (Haer-Wigman et al. 2015). The major types of non-syndromic RP are usually distinguished by their pattern of inheritance: autosomal dominant, autosomal recessive, or X-linked.

Initial evidence that mutations to the MERTK gene played a role in retinal dystrophy came from D'Cruz and collaborators (2000) who identified a small deletion that disrupted the MERTK gene in the DNA from the Royal College of Surgeons (RCS) rat. These investigators implicated this MERTK mutation in the abnormal RPE cell phagocytosis of photoreceptor outer segments (Bourne et al. 1938), a functional lesion thought to cause postnatal loss of photoreceptors in the RCS rat. In support of these findings, when a viral vector was used to transfer wild-type MERTK to the RPE of RCS rats, the RPE phagocytosis defect was resolved and photoreceptors were preserved in the areas of the retina that were transduced with the viral construct (Vollrath et al. 2001). Additional evidence supporting the role of MERTK gene mutation in retinal dystrophy came from a study of mice that were homozygous for a targeted disruption of the MERTK receptor tyrosine kinase gene (mer^{kd}). As in the RSC rat, MERTK protein was not expressed in the tissues of mer^{kd} mice, and the retinal phenotype of the mer^{kd} mice was quite similar to that of the RCS rat with defective RPE phagocytosis and progressive thinning of the outer nuclear layer; by P45 mer^{kd} mice retained only a single row of photoreceptor nuclei

(Duncan et al. 2003). Recently the results of Vollrath in RCS rats have been replicated. Conlon and collaborators (2013) using an AAV2 vector expressing human MERTK cDNA driven by an RPE specific VMD2 promotor demonstrated preserved ERG activity in the RCS rat when treatment was administered on P9 and assessments were performed 2 months posttreatment. LaVail et al. (2016) extended these observations in RCS rats to 6.5 months; at this time point, photoreceptor nuclei in the ONL of the untreated eye were reduced to less than a single row, in striking contrast to the near normal appearance of the ONL in the area of maximal retina protection in the vector-treated animals. A similar extent of photoreceptor preservation was observed with this viral construct in mer^{kd} mice that were followed for about 50 days following treatment (LaVail et al. 2016).

Based on the phenotypic similarity between the RCS rat, mer^{kd} mice, and patients with the MER gene mutation, and the effects of AAV gene augmentation in animal models, a phase 1 clinical trial of an AAV2 vector expressing human MERTK cDNA driven by an RPE specific VMD2 promotor was initiated in patients with MERTK-associated retinal disease (NCT01482195). Patient recruitment began after completion of a 6-month study in primates in which there were no dose-limiting adverse findings in animals receiving 10 and 50 μl of 10^{12} vg/100 μl. In the Phase 1 clinical study, six patients were followed for up to 2 years after treatment. The age of these patients ranged from 14 to 54 years and baseline visual acuity ranged between 20/50 and less than 20/6,400. The worse-seeing eye in five patients received a subretinal injection of 4×10^{10} vg/100 μl; the first two patients received a 150 μl injection and the remaining four patients received a 450 μl injection. At the 2-year time point, none of the patients developed complications that could be attributed to the gene vector. Adverse effects included keratitis in one patient and cataract formation in two patients; increased levels of AAV antibodies were observed in two patients, but neither was positive for rAAV vector genomes. Three patients had measurable improvements in visual acuity, but these improvements were lost in two of these patients by year 2 (Ghazi et al. 2016).

2.7 ND4 Gene Therapy

LHON is a maternal IRD thought to be caused by mutations in mitochondrial DNA. Disease presentation in LHON patients is characterized by the severe loss of central vision associated with the loss of retinal ganglion cells (RGCs); these changes typically develop in the second and third decades of life. Males are 4–5 times more likely to be symptomatic than females, but neither gender nor the specific gene mutation appears to influence the timing and severity of the initial visual loss (Man et al. 2002). While the prevalence of LHON is not well known, the disease is reported to occur in about 1 in 30,000 to 1 in 50,000 individuals (Man et al. 2003; Puomila et al. 2007). Although approximately 45 mutations have been linked to LHON, all of the mutations identified in symptomatic individuals are secondary to amino-acid substitutions in the mitochondrial DNA coding for the respiratory chain subunits of the nicotinamide adenine dinucleotide ubiquinone oxidoreductase

(complex I). Of the six mutations localized within this complex, the mutation in polypeptides ND1 (G3460A), ND4 (G11778A), and ND6 (T14484C) account for approximately 95% of all cases (Kirches 2011); mutation G11778 in ND4 appears to be the most common, accounting for 50–70% of all LHON. These latter mutations cause a defect in complex I function, resulting in the loss of energetic efficiency, increased oxidative stress, and an increase in the risk for apoptotic cell death. Perhaps the unique structure of RGCs, with their projection of axons for long distances from the stroma, makes them particularly sensitive to mitochondrial defects; RGC loss is the only clinically relevant phenotype in most LHON patients. No other major neuronal losses have been detected in affected retinae, even though the requirement of photoreceptor cells for ATP generation by oxidative phosphorylation (OXPHOS) is very high. These observations suggest that the mutation to complex 1 causes a relatively mild biochemical defect which leads to cell death only in RGCs (Carelli et al. 2009).

Although the mechanism is not understood, spontaneous visual improvement has been reported in some individuals with the T14484C mutation in ND6 (Lin, Pang et al. 2012). Heteroplasmy, a mixture of mutated and (normal) mitochondrial DNA, which occurs in 10–15% of individuals with LHON, appears to affect the risk for developing LHON in asymptomatic individuals, as well as the risk for transmission of the symptomatic disease (Chinnery et al. 2001). Heteroplasmy tends to progress toward homoplasmy in successive generations (Smith et al. 1993). Considerations of heteroplasmy and spontaneous visual improvement may become important in gene therapy trials.

Confirmation that the mitochondrial gene mutations affect mitochondrial function was first established in studies with transmitochondrial cybrid constructs. These constructs were created by enucleating cells from lymphoid cells lines established from LHON patients with the individual mitochondrial gene mutations. These cytoplasts were then fused with cells that lack mitochondrial DNA, i.e., the WAL-2A-p0 cell line. Early studies using complex I enzymatic assays on the cybrid constructs demonstrated that the human ND1 G3460A mutation resulted in a 79% reduction in specific activity and the ND4 G11778A mutation resulted in a 20% reduction, while the ND6 T14484C mutation did not affect complex I activity (Brown et al. 2000). Subsequent studies of cybrids have documented partial complex I and site I respiration defects, reduced ATP production and increased mitochondrial reactive oxygen species (ROS) production; all of these abnormalities have been implicated in RGC death evident in individuals with LHON (Baracca et al. 2005; Sala et al. 2008).

The next challenge to developing a treatment for LHON was to create a mouse model. While the use of viral vectors has made it possible to transduce foreign genes into ocular tissues, introducing foreign gene into the mitochondria of retinal tissues was considerably more complex. This was accomplished independently by several groups through their use of allotropic expression in which a nuclear encoded version of a gene normally encoded by mitochondrial DNA specified that the protein product would be expressed in the cytoplasm and then imported into the mitochondria. With this approach, Qi and collaborators (2007) created a mouse model in which the

mutant human ND4 subunit of complex I, containing an arginine-to-histidine substitution at amino acid 340, was transduced into the mitochondria of mouse RGCs. In these animals, histological evidence of optic nerve head (ONH) swelling was evident within 1 month of gene transduction. By 6 months, ONH swelling was displaced by disrupted mitochondrial cytoarchitecture, as well as optic nerve and RGC atrophy; these changes were accompanied by a threefold increase in ROS derived reaction products evident in histological tissue sections.

Lin, Sharpley et al. (2012) used a similar procedure to create a mouse LHON model by introducing the murine equivalent of the human ND6 mutation into mice RGC mitochondria. Animals with the ND6 mutation developed reduced retinal function, as assessed with ERG analysis; an age-related decline in central smaller caliber optic nerve fibers, with sparing of larger periheral fibers; neuronal accumulation of abnormal mitochondria; axonal swelling and axonal demyelination. Mitochondrial analysis revealed partial complex 1 and respiration defects, as well as increased ROS production.

Using the LHON mouse model with the human ND4 mutant, Koilkonda and collaborators (2014) attempted to rescue retinal structure and function by using a gene that directed a copy of the normal human ND4 gene to mouse RGC mitochondria; this construct was identified as ND4 ScAAV2-P1ND4v2. When this normal gene was allotopically expressed in the mouse RGC mitochondria, histology and ultrastructural analyses confirmed that the loss of RGCs and axons was prevented.

Ellouze and colleagues (2008) created a rat model of LHON by using in vivo electroporation to introduce the human ND4 mutant gene into rat eyes. As observed by the Qi group and the Lin group (Qi et al. 2007; Lin et al. 2012), expression of the mutant gene in RGC mitochondria induced the degeneration of RGCs; in the rat model RGC abundance was reduced by about 40% compared to control eyes.

To assess whether the mitochondrial co-expression of the normal ND4 gene could suppress the effects of the mutant gene in the electroporation human mutant ND4 model, Cwerman-Thibault and colleagues (2015) treated these animals with a viral construct of normal human ND4 that contains the *cis*-acting elements of the human *COX10* mRNA. These elements of the human *COX10* mRNA allowed the allotropic expression of the normal ND4 in rodent RGC mitochondria. In agreement with the results from Koilkonda and collaborators (2014), this construct of a normal ND4 gene prevented RGC and optic nerve degeneration and preserved complex I function and visual function.

Based on these studies in animal model of LHON, the therapeutic viral constructs tested by Koilkonda and colleagues (2014), ScAAV2-P1ND4v2, and by Cwerman-Thibault and colleagues (2015), AAV2/2-ND4, have advanced into clinical studies, NCT02161380 and NCT02064569, respectively. Initial results from study NCT02161380 that treated five LHON patients carrying the G11778A mutation with ScAAV2-P1ND4v2 have been reported (Feuer et al. 2016). At the 3-month time point, none of the patients lost vision and only minor adverse events were reported; these consisted of a transient increase of intraocular pressure (IOP), keratitis, subconjunctival hemorrhage, and a sore throat; a transient increase in

neutralizing antibodies against AAV2 in 1 participant was also reported. All blood samples were negative for vector DNA. Visual acuity in two of the five patients improved from hand motion to +7 letters and +15 letters, respectively.

GenSight Biologics is sponsoring the clinical development of AAV2/2-ND4. Recently GenSight (www.gensight-biologics.com) released the topline result from their phase 2 safety study in which cohorts of three patients received escalating doses of AAV2/2-ND4, identified as GS010. The average disease duration of patients participating in the study was 6 years. In the subpopulation of patients with a disease duration of less than 2 years, BCVA increased by 30 letters in the treated eye and by 13 letters in the untreated eye. Encouraged by these preliminary cone results, GenSight has initiated two phase 3 studies, NCT02652767 and NCT02652780.

2.8 CNGA3 and CNGB3 Gene Therapies

Visual signal transduction is initiated by the photoisomerization of rhodopsin, i.e., the conversion of 11-cis retinal to all-trans retinal. This isomerization changes the conformation of the opsin g-protein coupled receptor, initiating a signal transduction cascade that activates phosphodiesterase, lowering the concentration of cGMP. Decreasing cGMP levels causes closure of cyclic GMP-gated cation channels leading to hyperpolarization of the photoreceptor cell. This hyperpolarization results in the closure of voltage-gated calcium channels leading to a decreased release of the neurotransmitter glutamate.

Cyclic GMP-gated channels consists of four subunits around a central pore. In cone photoreceptors, the subunits consist of two copies of CNGA3 and two copies of CNGB3 (Peng et al. 2004). Mutations to the genes expressing CNGA3 and CNGB3 have been linked to autosomal recessive congenital achromatopsia, characterized by the absence of color vision and ERG cone response, and the presence of photophobia and nystagmus or a history of nystagmus; typically rod photoreceptor function remains normal or near normal. The retina phenotypes secondary to mutations to CNGB3 and CNGA3 are indistinguishable. Mutations in the genes for CNGB3 and CNGA3 together account for about 75% of all cases of achromatopsia; mutations to the CNGB3 gene appear to be more prevalent, accounting for about 50% of patients with achromatopsia. The prevalence of achromatopsia is about 1 in 30,000 individuals (Kohl et al. 2000; Wissinger et al. 2001; Kohl et al. 2005; Khan et al. 2007).

Proof-of-concept of gene augmentation therapy in achromatopsia has been established in the Cnga3$^{-/-}$ mouse model using an AAV5 vector containing the normal mouse CGNA3 gene under control of the blue opsin promotor (Michalakis et al. 2010). In this study, treatment was administered to 12- to 14-day-old CNGA3$^{-/-}$ mice. Following successful viral transduction, CNGA3 protein was expressed in cone photoreceptors; ERG assessments performed 10 weeks post-surgery demonstrated restoration of cone function and histology demonstrated preserved retinal structure. Photopic visual function was assessed in a correct-

choice water maze; wild-type mice and Cngb3$^{-/-}$ treated mice correctly completed the maze 76.4% and 73.8% of the time, respectively, while untreated Cngb3$^{-/-}$ mice successfully completed the maze only 55.6% of the time.

Proof-of-concept has also been established in a strain of sheep that do not express the CNGA3 gene. When these animals were treated with an AAV5 vector containing either the normal mouse or normal human CGNA3 gene, under control of the red/green opsin promotor (Banin et al. 2015), ERG recordings showed marked long-term improvement in cone function; ERG assessments performed 6 months post-surgery in animals receiving vehicle alone failed to show any improvement. Postoperative visual behavior assessments using a two-barrier maze were performed beginning 1–2 months after treatment. Under scotopic conditions, maze performance by CNGA3 mutant sheep was similar to that of unaffected sheep. Under photopic conditions, however, unaffected sheep and CNGA3 mutant animals receiving gene augmentation therapy completed the maze in 5.6 and 7.4–8.2 s, respectively, while untreated CNGA3 mutant sheep completed the test in 25.6 s. The completion times in CNGA3 mutant animals receiving gene therapy were not significantly different between animals receiving the mouse or human gene; the level of performance observed in the CNGA3 mice receiving gene therapy was maintained for more than 3 years postoperatively.

Proof-of-concept for CNGB3 gene augmentation has been established in Cngb3$^{-/-}$ mice (Carvalho et al. 2011), using an AAV2/8 viral vector containing the human CNGB3 cDNA under control of the human cone arrestin promotor. Proof-of-concept has also been established in two canine models of CNGB3 achromatopsia. In the first model, CNGB3$^{-/-}$, achromatopsia is a consequence of a missense mutation in CNGB3. In the second, achromatopsia is a consequence of a genomic deletion of the entire gene, CNGB3$^{m/m}$ (Komaromy et al. 2010). These latter studies in dogs used an AAV5 vector containing the normal human CGNB3 gene, under control of the red opsin promotor.

Following successful gene transduction in the Cngb3$^{-/-}$ mice, cone specific expression of CNGB3 was demonstrated in both M- and S-cones. When compared to the untreated eye, cone density in the treated eye compared with the untreated eye was significantly ($p < 0.01$) protected 8 weeks after treatment when treatment was administered at P15. Treatment at P15 and P30 achieved near complete ERG restoration of cone function, i.e., 90% of wild-type level; treatment at P90 and P180 also rescue ERG, but at 70–60% of wild-type levels, respectively.

In both canine models, a single subretinal injection if the AAV5 vector restored cone function, as assessed with ERG. However, the restoration of cone function appeared to be dependent upon the age of the animals at the time of treatment. One of three animals demonstrated a restoration of function when treatment was administered to dogs over 54 weeks of age; in contrast, 11 of 14 dogs exhibited cone rescue when treatment was administered to animals younger than 28 weeks of age. Postoperative visual behavior was assessed using an obstacle avoidance course. Under photopic conditions, normal control dogs completed the course in about 5 s while untreated dogs with the CNGB3 mutation completed the course in

about 25 s. Dogs with the either of the two CNGB3 mutations, receiving vector treatment, completed the course in approximately 7–8 s.

These studies have provided the rationale for Applied Genetic Technologies Corporation to initiate a phase 1/2 study of a recombinant AAV2 viral construct of the human CNGB3 (rAAV2tYF-PR1.7-hCNGB3) in patients with CNGB3 mutations (NCT02599922) and for STZ Eyetrial to initiate a phase 1/2 study of a recombinant AAV viral construct of human CNGA3 (rAAV.hCNGA3) in patients with CNGB3 mutations (NCT02610582). The primary endpoint for both studies is safety, and the key secondary endpoint is improved visual function.

Acknowledgments The Foundation Fighting Blindness, Columbia, Maryland, was extremely helpful during the writing of this review. Brian Mansfield, PhD and Stephen Rose, PhD generously shared their extensive knowledge of inherited retinal dystrophies and Brian Mansfield, PhD critically reviewed the manuscript.

References

Abu-Safieh L, Alrashed M, Anazi S, Alkuraya H, Khan AO, Al-Owain M, Al-Zahrani J, Al-Abdi L, Hashem M, Al-Tarimi S (2013) Autozygome-guided exome sequencing in retinal dystrophy patients reveals pathogenetic mutations and novel candidate disease genes. Genome Res 23:236–247

Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J (2001) Gene therapy restores vision in a canine model of childhood blindness. Nat Genet 28(1):92–95

Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, Bennicelli J, Dejneka NS, Pearce-Kelling SE, Maguire AM, Palczewski K, Hauswirth WW, Jacobson SG (2005) Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Mol Ther 12(6):1072–1082

Aguirre GD, Baldwin V, Pearce-Kelling S, Narfstrom K, Ray K, Acland GM (1998) Congenital stationary night blindness in the dog: common mutation in the RPE65 gene indicates founder effect. Mol Vis 4:23–29

Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 277(5333):1805–1807

Allocca M, Doria M, Petrillo M, Colella P, Garcia-Hoyos M, Gibbs D, Kim SR, Maguire A, Rex TS, Di Vicino U, Cutillo L, Sparrow JR, Williams DS, Bennett J, Auricchio A (2008) Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice. J Clin Invest 118(5):1955–1964

Anand V, Barral DC, Zeng Y, Brunsmann F, Maguire AM, Seabra MC, Bennett J (2003) Gene therapy for choroideremia: in vitro rescue mediated by recombinant adenovirus. Vision Res 43:919–926

Bainbridge JW, Mehat MS, Sundaram V, Robbie SJ, Barker SE, Ripamonti C, Georgiadis A, Mowat FM, Beattie SG, Gardner PJ, Feathers KL, Luong VA, Yzer S, Balaggan K, Viswanathan A, de Ravel TJ, Casteels I, Holder GE, Tyler N, Fitzke FW, Weleber RG, Nardini M, Moore AT, Thompson DA, Petersen-Jones SM, Michaelides M, van den Born LI, Stockman A, Smith AJ, Rubin G, Ali RR (2015) Long-term effect of gene therapy on Leber's congenital amaurosis. N Engl J Med 372:1887–1897

Banin E, Gootwine E, Obolensky A, Ezra-Elia R, Ejzenberg A, Zelinger L, Honig H, Rosov A, Yamin E, Sharon D, Averbukh E, Hauswirth WW, Ofri R (2015) Gene augmentation therapy

restores retinal function and visual behavior in a sheep model of CNGA3 achromatopsia. Mol Ther 23(9):1423–1433

Baracca A, Solaini G, Sgarbi G, Lenaz G, Baruzzi A, Schapira AHV, Martinuzzi A, Carelli V (2005) Severe impairment of complex I-driven adenosine triphosphate synthesis in Leber hereditary optic neuropathy cybrids. Arch Neurol 62(5):730–736

Bennett J, Ashtari M, Wellman J, Marshall KA, Cyckowski LL, Chung DC, McCague S, Pierce EA, Chen Y, Bennicelli JL, Zhu X, Ying G-S, Sun J, Wright JF, Auricchio A, Simonelli F, Shindler KS, Mingozzi F, High KA, Maguire AM (2012) AAV2 gene therapy readministration in three adults with congenital blindness. Sci Transl Med 4(120):120ra15

Bennicelli J, Wright JF, Komaromy A, Jacobs JB, Hauck B, Zelenaia O, Mingozzi F, Hui D, Chung D, Rex TS, Wei Z, Qu G, Zhou S, Zeiss C, Arruda VR, Acland GM, Dell'Osso LF, High KA, Maguire AM, Bennett J (2008) Reversal of blindness in animal models of Leber congenital amaurosis using optimized AAV2-mediated gene transfer. Mol Ther 16(3):458–465

Berson EL (2007) Long-term visual prognoses in patients with retinitis pigmentosa: the Ludwig von Sallmann lecture. Exp Eye Res 85(1):7–14

Binley K, Widdowson P, Loader J, Kelleher M, Iqball S, Ferrige G, de Belin J, Carlucci M, Angell-Manning D, Hurst F, Ellis S, Miskin J, Fernandes A, Wong P, Allikmets R, Bergstrom C, Aaberg T, Yan J, Kong J, Gouras P, Prefontaine A, Vezina M, Bussieres M, Naylor S, Mitrophanous KA (2013) Transduction of photoreceptors with EIAV lentiviral vectors; safety and biodistribution of StarGen for Stargardt disease. Invest Ophthalmol Vis Sci 54 (6):4061–4071

Black A, Vasireddy V, Chung DC, Maguire AM, Gaddameedi R, Tolmachova T, Seabra M, Bennett J (2014) Adeno-associated virus 8-mediated gene therapy for choroideremia: preclinical studies in in vitro and in vivo models. J Gene Med 16:122–130

Bourne MC, Campbell DA, Tansley K (1938) Hereditary degeneration of the rat retina. Br J Ophthalmol 22(10):613–623

Braun TA, Mullins RF, Wagner AH, Andorf JL, Johnston RM, Bakall BB, Deluca AP, Fishman GA, Lam BL, Weleber RG, Cideciyan AV, Jacobson SG, Sheffield VC, Tucker BA, Stone EM (2013) Non-exomic and synonymous variants in ABCA4 are an important cause of Stargardt disease. Hum Mol Genet 22(25):5136–5145

Brown MD, Trounce IA, Jun AS, Allen JC, Wallace DC (2000) Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation. J Biol Chem 275(51):39831–39836

Cai X, Conley SM, Naash MI (2009) RPE65: role in the visual cycle, human retinal disease, and gene therapy. Ophthalmic Genet 30(2):57–62

Carelli V, La Morgia C, Valentino ML, Barboni P, Ross-Cisneros FN, Sadun AA (2009) Retinal ganglion cell neurodegeneration in mitochondrial inherited disorders. Biochim Biophys Acta 1787(5):518–528

Carvalho LS, Xu J, Pearson RA, Smith AJ, Bainbridge JW, Morris LM, Fliesler SJ, Ding X-Q, Ali RR (2011) Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Hum Mol Genet 20(16):3161–3175

Chacon-Camacho OF, Zenteno JC (2015) Review and update on the molecular basis of Leber congenital amaurosis. World J Clin Cases 3(2):112–124

Chang B, Hawes NL, Hurd RE, Davisson MT, Nusinowitz S, Heckenlively JR (2002) Retinal degeneration mutants in the mouse. Vision Res 42(4):517–525

Charbel P, Bolz HJ, Ebermann I, Domeier E, Holz FG, Scholl HP (2009) Characterization of severe rod-cone dystrophy in a consanguineous family with a splice site mutation in the MERTK gene. Br J Ophthalmol 93:920–925

Chinnery PF, Andrews RM, Turnbull DM, Howell NN (2001) Leber hereditary optic neuropathy: does heteroplasmy influence the inheritance and expression of the G11778A mitochondrial DNA mutation? Am J Med Genet 98:235–243

Cideciyan AV, Jacobson SG, Beltran WA, Sumaroka A, Swider AM, Iwabe S, Roman AJ, Olivares MB, Schwartz SB, Komaromy AM, Hauswirth WW, Aguirre GD (2013) Human

retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. Proc Natl Acad Sci U S A 110(6):E517–E525

Conlon TJ, Deng WT, Erger K, Cossette T, Pang J, Ryals R, Clement N, Cleaver B, McDoom I, Boye SE, Peden MC, Sherwood MB, Abernathy CR, Alkuraya FS, Boye SL, Hauswirth WW (2013) Preclinical potency and safety studies of an AAV2-mediated gene therapy vector for the treatment of MERTK associated retinitis pigmentosa. Hum Gene Ther Clin Dev 24(1):23–28

Cwerman-Thibault H, Augustin S, Lechauve C, Ayache J, Ellouze S, Sahel JA, Corral-Debrinski M (2015) Nuclear expression of mitochondrial ND4 leads to the protein assembling in complex I and prevents optic atrophy and visual loss. Mol Ther Methods Clin Dev 2:15003. doi:10.1038/mtm.2015.3

D'Cruz PM, Yasumura D, Weir J, Matthes MT, Abderrahim H, LaVail MM, Vollrath D (2000) Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. Hum Mol Genet 9(4):645–651

Dong B, Nakai H, Xiao W (2010) Characterization of genome integrity for oversized recombinant AAV vector. Mol Ther 18(1):87–92

Dryja TP, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DW, Sandberg MA, Berson EL (1990) A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature 343 (6256):364–366

Duan D, Yue Y, Engelhardt JF (2001) Expanding AAV packaging capacity with trans-splicing or overlapping vectors: a quantitative comparison. Mol Ther 4(4):383–391

Duncan JL, LaVail MM, Yasumura D, Matthes MT, Yang H, Trautmann N, Chappelow AV, Feng W, Earp WS, Matsushima GK, Vollrath D (2003) An RCS-like retinal dystrophy phenotype in mer knockout mice. Invest Ophthalmol Vis Sci 44:826–838

Dyka FM, Boye SL, Chiodo VA, Hauswirth WW, Boye SE (2014) Dual adeno-associated virus vectors result in efficient in vitro and in vivo expression of an oversized gene, MYO7A. Hum Gene Ther Methods 25(2):166–177

Edwards TL, Jolly JK, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Black GC, Webster AR, Lotery AJ, Holder GE, Xue K, Downe SM, Simunovic MP, Seabra MC, MacLaren RE (2016) Visual acuity after retinal gene therapy for choroideremia. N Engl J Med 374 (20):1996–1998

Ellouze S, Augustin S, Bouaita A, Bonnet C, Simonutti M, Forster V, Picaud S, Sahel JA, Corral-Debrinski M (2008) Optimized allotopic expression of the human mitochondrial ND4 prevents blindness in a rat model of mitochondrial dysfunction. Am J Hum Genet 83(3):373–387

Farber DB, Lolley RN (1976) Enzymic basis for cyclic GMP accumulation in degenerative photoreceptor cells of mouse retina. J Cyclic Nucleotide Res 2(3):139–148

Feathers KL, Lyubarsky AL, Khan NW, Teofilo K, Swaroop A, Williams DS, Pugh EN Jr, Thompson DA (2008) Nrl-knockout mice deficient in Rpe65 fail to synthesize 11-cis retinal and cone outer segments. Invest Ophthalmol Vis Sci 49(3):1126–1135

Feuer WJ, Schiffman JC, Davis JL, Porciatti V, Gonzalez P, Koilkonda RD, Yuan H, Lalwani A, Lam BL, Guy J (2016) Gene therapy for Leber hereditary optic neuropathy: initial results. Ophthalmology 123(3):558–570

Freund PR, Sergeev YV, MacDonald IM (2016) Analysis of a large choroideremia dataset does not suggest a preference for inclusion of certain genotypes in future trials of gene therapy. Mol Genet Genomic Med 4(3):344–358

Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, Apfelstedt-Sylla E, Vollrath D (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nat Genet 26:270–271

Ghazi NG, Abboud EB, Nowilaty SR, Alkuraya H, Alhommadi A, Cai H, Hou R, Deng W-T, Boye SL, Almaghamsi A, Saikhan FA, Al-Dhibi H, Birch D, Chung C, Colak D, LaVail MM, Vollrath D, Erger K, Wang W, Conlon T, Zhang K, Hauswirth W, Alkuraya FS (2016) Treatment of retinitis pigmentosa due to MERTK mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum Genet 135(3):327–343

Ghosh A, Yue Y, Lai Y, Duan D (2008) A hybrid vector system expands adeno-associated viral vector packaging capacity in a transgene-independent manner. Mol Ther 16(1):124–130

Gibbs D, Kitamoto J, Williams DS (2003) Abnormal phagocytosis by retinal pigmented epithelium that lacks myosin VIIa, the Usher syndrome 1B protein. Proc Natl Acad Sci U S A 100 (11):6481–6486

Gibson F, Walsh J, Mburu P, Varela A, Brown KA, Antonio M, Beisel KW, Steel KP, Brown SD (1995) A type VII myosin encoded by the mouse deafness gene shaker-1. Nature 374 (6517):62–64

Graham DK, Dawson TL, Mullaney DL, Snodgrass HR, Earp HS (1994) Cloning and mRNA expression analysis of a novel human protooncogene, c-mer. Cell Growth Differ 5(6):647–657

Haer-Wigman L, Newman H, Leibu R, Bax NM, Baris HN, Rizel L, Banin E, Massarweh A, Roosing S, Lefeber DJ, Zonneveld-Vrieling MN, Isakov O, Shomron N, Sharon D, Den Hollander AI, Hoyng CB, Cremers FPM, Ben-Yosef T (2015) Non-syndromic retinitis pigmentosa due to mutations in the mucopolysaccharidosis type IIIC gene, heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT). Hum Mol Genet 24(13):3742–3751

Hafezi F, Grimm C, Simmen B, Wenzel A, Reme C (2000) Molecular ophthalmology: an update on animal models for retinal degenerations and dystrophies. Br J Ophthalmol 84(8):922–927

Hashimoto T, Gibbs D, Lillo C, Azarian SM, Legacki E, Zhang XM, Yang XJ, Williams DS (2007) Lentiviral gene replacement therapy of retinas in a mouse model for Usher syndrome type 1B. Gene Ther 14(7):584–594

Jacobson SG, Boye SL, Aleman TS, Conlon TJ, Zeiss CJ, Roman AJ, Cideciyan AV, Schwartz SB, Komaromy AM, Doobrajh M, Cheung AY, Sumaroka A, Pearce-Kelling SE, Aguirre GD, Kaushal S, Maguire AM, Flotte TR, Hauswirth WW (2006) Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in Leber congenital amaurosis. Hum Gene Ther 17(8):845–858

Jacobson SG, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, Peden MC, Aleman TS, Boye SL, Sumaroka A, Conlon TJ, Calcedo R, Pang JJ, Erger KE, Olivares MB, Mullins CL, Swider M, Kaushal S, Feuer WJ, Iannaccone A, Fishman GA, Stone EM, Byrne BJ, Hauswirth WW (2012) Gene therapy for Leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Arch Ophthalmol 130(1):9–24

Jacobson SG, Cideciyan AV, Roman AJ, Sumaroka A, Schwartz SB, Heon E, Hauswirth WW (2015) Improvement and decline in vision with gene therapy in childhood blindness. N Engl J Med 372:1920–1926

Janssen A, Min SH, Molday LL, Tanimoto N, Seeliger MW, Hauswirth WW, Molday RS, Weber BH (2008) Effect of late-stage therapy on disease progression in AAV-mediated rescue of photoreceptor cells in the retinoschisin-deficient mouse. Mol Ther 16(6):1010–1017

Kaminski WE, Piehler A, Wenzel JJ (2006) ABC A-subfamily transporters: structure, function and disease. Biochim Biophys Acta 1762(5):510–524

Khan NW, Wissinger B, Kohl S, Sieving PA (2007) CNGB3 achromatopsia with progressive loss of residual cone function and impaired rod-mediated function. Invest Ophthalmol Vis Sci 48:3864–3871

Kimberling WJ, Hildebrand MS, Shearer AE, Jensen ML, Halder JA, Trzupek K, Cohn ES, Weleber RG, Stone EM, Smith RJ (2010) Frequency of Usher syndrome in two pediatric populations: implications for genetic screening of deaf and hard of hearing children. Genet Med 12:512–516

Kirches E (2011) LHON: mitochondrial mutations and more. Curr Genomics 12:44–54

Kjellstrom S, Bush RA, Zeng Y, Takada Y, Sieving PA (2007) Retinoschisin gene therapy and natural history in the Rs1h-KO mouse: long-term rescue from retinal degeneration. Invest Ophthalmol Vis Sci 48:3837–3845

Kohl S, Baumann B, Broghammer M, Jagle H, Sieving P, Kellner U, Spegal R, Anastasi M, Zrenner E, Sharpe LT, Wissinger B (2000) Mutations in the CNGB3 gene encoding the beta-

subunit of the cone photoreceptor cGMP-gated channel are responsible for achromatopsia (ACHM3) linked to chromosome 8q21. Hum Mol Genet 9:2107–2116

Kohl S, Varsanyi B, Antunes GA, Baumann B, Hoyng CB, Jägle H, Rosenberg T, Kellner U, Lorenz B, Salati R, Jurklies B, Farkas A, Andreasson S, Weleber RG, Jacobson SG, Rudolph G, Castellan C, Dollfus H, Legius E, Anastasi M, Bitoun P, Lev D, Sieving PA, Munier FL, Zrenner E, Sharpe LT, Cremers FP, Wissinger B (2005) CNGB3 mutations account for 50% of all cases with autosomal recessive achromatopsia. Eur J Hum Genet 13 (3):302–308

Koilkonda R, Yu H, Talla V, Porciatti V, Feuer WJ, Hauswirth WW, Chiodo V, Erger KE, Boye SL, Lewin AS, Conlon TJ, Renner L, Neuringer M, Detrisac C, Guy J (2014) LHON gene therapy vector prevents visual loss and optic neuropathy induced by G11778A mutant mitochondrial DNA: biodistribution and toxicology profile. Invest Ophthalmol Vis Sci 55 (12):7739–7753

Komaromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, Tanaka JC, Acland GM, Hauswirth WW, Aguirre GD (2010) Gene therapy rescues cone function in congenital achromatopsia. Hum Mol Genet 19:2581–2593

Kong J, Kim SR, Binley K, Pata I, Doi K, Mannik J, Zernant-Rajang J, Kan O, Iqball S, Naylor S, Sparrow JR, Gouras P, Allikmets R (2008) Correction of the disease phenotype in the mouse model of Stargardt disease by lentiviral gene therapy. Gene Ther 15(19):1311–1320

Lai Y, Yue Y, Duan D (2010) Evidence for the failure of adeno-associated virus serotype 5 to package a viral genome > or = 8.2 kb. Mol Ther 18(1):75–79

LaVail MM, Yasumura D, Matthes MT, Yang H, Hauswirth WW, Deng WT, Vollrath D (2016) Gene therapy for MERTK-associated retinal degenerations. Adv Exp Med Biol 854:487–493

Le Meur G, Stieger K, Smith AJ, Weber M, Deschamps JY, Nivard D, Mendes-Madeira A, Provost N, Péréon Y, Cherel Y, Ali RR, Hamel C, Moullier P, Rolling F (2007) Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. Gene Ther 14:292–303

Lentz J, Keats BJB (2016) Usher syndrome type I. In: Pagon RA, Adam MP, Ardinger HH et al (eds) GeneReviews® [Internet]. University of Washington, Seattle, pp 1993–2016 10 Dec 1999 [Updated 19 May 2016]

Li J, Sun W, Wang B, Xiao X, Liu XQ (2008) Protein trans-splicing as a means for viral vector-mediated in vivo gene therapy. Hum Gene Ther 19(9):958–964

Li Q, Miller R, Han PY, Pang J, Dinculescu A, Chiodo V, Hauswirth WW (2008) Intraocular route of AAV2 vector administration defines humoral immune response and therapeutic potential. Mol Vis 14:1760–1769

Li W, Kong F, Li X, Dai X, Liu X, Zheng Q, Wu R, Zhou X, Lü F, Chang B, Li Q, Hauswirth WW, Qu J, Pang JJ (2009) Gene therapy following subretinal AAV5 vector delivery is not affected by a previous intravitreal AAV5 vector administration in the partner eye. Mol Vis 15:267–275

Libby RT, Steel KP (2001) Electroretinographic anomalies in mice with mutations in Myo7a, the gene involved in human Usher syndrome type 1B. Invest Ophthalmol Vis Sci 42(3):770–778

Lin CS, Sharpley MS, Fan W, Waymire KG, Sadun AA, Carelli V, Ross-Cisneros FN, Baciu P, Sung E, McManus MJ, Pan BX, Gil DW, Macgregor GR, Wallace DC (2012) Mouse mtDNA mutant model of Leber hereditary optic neuropathy. Proc Natl Acad Sci U S A 109 (49):20065–20070

Lin H-Z, Pang C-Y, Chen S-P, Tsai R-K (2012) Vision improvement in a Taiwanese (Han Chinese) family with Leber's hereditary optic neuropathy. Kaohsiung J Med Sci 28:679–682

Liu X, Ondek B, Williams DS (1998) Mutant myosin VIIa causes defective melanosome distribution in the RPE of shaker-1 mice. Nat Genet 19(2):117–118

Liu X, Udovichenko IP, Brown SD, Steel KP, Williams DS (1999) Myosin VIIa participates in opsin transport through the photoreceptor cilium. J Neurosci 19(15):6267–6274

MacDonald IM, Mah DY, Ho YK, Lewis RA, Seabra MC (1998) A practical diagnostic test for choroideremia. Ophthalmology 105(9):1637–1640

MacDonald IM, Hume S, Chan S, Sebra MC (2003) Choroideremia. In: Pagon RA, Adam MP, Ardinger HH et al (eds) GeneReviews® [Internet]. University of Washington, Seattle, pp 1993–2016

Mackay DS, Henderson RH, Sergouniotis PI, Li Z, Moradi P, Holder GE, Waseem N, Bhattacharya SS, Aldahmesh MA, Alkuraya FS, Meyer B, Webster AR, Moore AT (2010) Novel mutations in MERTK associated with childhood onset rod- cone dystrophy. Mol Vis 16:369–377

MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, Clark KR, During MJ, Cremers FPM, Black GCM, Lotery AJ, Downes SM, Webster AR, Seabra MC (2014) Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet 383(9923):1129–1137

Maeda A, Maeda T, Golczak M, Palczewski K (2008) Retinopathy in mice induced by disrupted all-trans-retinal clearance. J Biol Chem 283(39):26684–26693

MaGuire W (2005) Human gene transfer protocol #740 – a Phase I safety study in subjects with Leber Congenital Amaurosis (LCA) using adeno-associated viral vector to deliver the gene for human RPE65 into the Retinal Pigment Epithelium (RPE). FDA OBA Presentation

Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF (2003) The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 72 (2):333–339

Man PYW, Turnbull DM, Chinnery PF (2002) Leber hereditary optic neuropathy. J Med Genet 39:162–169

Michalakis S, Mühlfriedel R, Tanimoto N, Krishnamoorthy V, Koch S, Fischer MD, Becirovic E, Bai L, Huber G, Beck SC, Fahl E, Buning H, Paquet-Durand F, Zong X, Gollisch T, Biel M, Seeliger MW (2010) Restoration of cone vision in the CNGA3−/− mouse model of congenital complete lack of cone photoreceptor function. Mol Ther 18(12):2057–2063

Molday LL, Hicks D, Sauer CG, Weber BH, Molday RS (2001) Expression of X-linked retinoschisis protein RS1 in photoreceptor and bipolar cells. Invest Ophthalmol Vis Sci 42:816–825

Molday RS, Kellner U, Weber BHF (2012) X-linked juvenile retinoschisis: Clinical diagnosis, genetic analysis, and molecular mechanisms. Prog Retin Eye Res 3:195–212 195e212

Narfstrom K, Vaegan, Katz M, Bragadottir R, Rakoczy EP, Seeliger M (2005) Assessment of structure and function over a 3-year period after gene transfer in RPE65-/- dogs. Doc Ophthalmol 111:39–48

Orhan E, Dalkara D, Neuillé M, Lechauve C, Michiels C, Picaud S, Léveillard T, Sahel JA, Naash MI, Lavail MM, Zeitz C, Audo I (2015) Genotypic and phenotypic characterization of P23H line 1 rat model. PLoS One 10(5):e0127319

Pang JJ, Chang B, Hawes NL, Hurd RE, Davisson MT, Li J, Noorwez SM, Malhotra R, McDowell JH, Kaushal S, Hauswirth WW, Nusinowitz S, Thompson DA, Heckenlively JR (2005) Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). Mol Vis 11:152–162

Park TK, Wu Z, Kjellstrom S, Zeng Y, Bush RA, Sieving PA, Colosi P (2009) Intravitreal delivery of AAV8 retinoschisin results in cell type-specific gene expression and retinal rescue in the Rs1-KO mouse. Gene Ther 16(7):916–926

Patel N, Aldahmesh MA, Alkuraya H, Anazi S, Alsharif H, Khan AO, Sunker A, Al-Mohsen S, Abboud EB, Nowilaty SR, Alowain M, Al-Zaidan H, Al-Saud B, Alasmari A, Abdel-Salam GM, Abouelhoda M, Abdulwahab FM, Ibrahim N, Naim E, Al-Younes B, E AlMostafa A, AlIssa A, Hashem M, Buzovetsky O, Xiong Y, Monies D, Altassan N, Shaheen R, Al-Hazzaa SA, Alkuraya FS (2015) Expanding the clinical, allelic, and locus heterogeneity of retinal dystrophies. Genet Med 18(6):554–562

Pearson RA, Hippert C, Graca AB, Barber AC (2014) Photoreceptor replacement therapy: challenges presented by the diseased recipient retinal environment. Vis Neurosci 31 (4–5):333–344

Peng C, Rich ED, Varnum MD (2004) Subunit configuration of heteromeric cone cyclic nucleotide-gated channels. Neuron 42:401–410
Peng YW, Zallocchi M, Wang WM, Delimont D, Cosgrove D (2011) Moderate light-induced degeneration of rod photoreceptors with delayed transducin translocation in shaker1 mice. Invest Ophthalmol Vis Sci 52(9):6421–6427
Petrs-Silva H, Linden R (2014) Advances in gene therapy technologies to treat retinitis pigmentosa. Clin Ophthalmol 8:127–136
Puomila A, Hämäläinen P, Kivioja S, Savontaus ML, Koivumäki S, Huoponen K, Nikoskelainen E (2007) Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. Eur J Hum Genet 15(10):1079–1089
Qi X, Sun L, Lewin AS, Hauswirth WW, Guy J (2007) The mutant human ND4 subunit of complex I induces optic neuropathy in the mouse. Invest Ophthalmol Vis Sci 48:1–10
Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, Goletz P, Ma JX, Crouch RK, Pfeifer K (1998) Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. Nat Genet 20(4):344–351
Reich SJ, Auricchio A, Hildinger M, Glover E, Maguire AM, Wilson JM, Bennett J (2003) Efficient trans-splicing in the retina expands the utility of adeno-associated virus as a vector for gene. Hum Gene Ther 14(1):37–44
Rohrer B, Goletz P, Znoiko S, Ablonczy Z, Ma J-X, Redmond TM, Crouch RK (2003) Correlation of regenerable opsin with rod ERG signal Rpe65-/- mice during development and aging. Invest Ophthalmol Vis Sci 44:310–315
Ross JW, Fernandez de Castro JP, Zhao J, Samuel M, Walters E, Rios C, Bray-Ward P, Jones BW, Marc RE, Wang W, Zhou L, Noel JM, McCall MA, DeMarco PJ, Prather RS, Kaplan HJ (2012) Generation of an inbred miniature pig model of retinitis pigmentosa. Invest Ophthalmol Vis Sci 53(1):501–507
Sahel J-A, Marazova K, Audo I (2015) Clinical characteristics and current therapies for inherited retinal degenerations. Cold Spring Harb Perspect Med. doi:10.1101/cshperspect.a017111
Sala G, Trombin F, Beretta S, Tremolizzo L, Presutto P, Montopoli M, Fantin M, Martinuzzi A, Carelli V, Ferrarese C (2008) Antioxidants partially restore glutamate transport defect in Leber hereditary optic neuropathy cybrids. J Neurosci Res 86(15):3331–3337
Sauer CG, Gehrig A, Warneke-Wittstock R, Marquardt A, Ewing CC, Gibson A, Lorenz B, Jurklies B, Weber BH (1997) Positional cloning of the gene associated with X-linked juvenile retinoschisis. Nat Genet 17:164–170
Schindler EI, Nylen EL, Ko AC, Affatigato LM, Heggen AC, Wang K, Sheffield VC, Stone EM (2010) Deducing the pathogenic contribution of recessive ABCA4 alleles in an outbred population. Hum Mol Genet 19(19):3693–3701
Smith KH, Johns DR, Heher KL, Miller NR (1993) Heteroplasmy in Leber's hereditary optic neuropathy. Arch Ophthalmol 111(11):1486–1490
Song D, Grieco S, Li Y, Hunter Chu AS, Zhao L, Song Y, DeAngelis RA, Shi LY, Liu Q, Pierce EA, Nishina PM, Lambris JD, Dunaief JL (2014) A murine rp1 missense mutation causes protein mislocalization and slowly progressive photoreceptor degeneration. Am J Pathol 184 (10):2721–2729
Sowden JC (2015) Developing stem cell therapy for retinal dystrophies. Cilia 4(Suppl 1):O19
Sun H, Nathans J (2000) ABCR: rod photoreceptor-specific ABC transporter responsible for Stargardt disease. Methods Enzymol 315:879–897
Tada A, Wada Y, Sato H, Itabashi T, Kawamura M, Tamai M, Nishida K (2006) Screening of the MERTK gene for mutations in Japanese patients with autosomal recessive retinitis pigmentosa. Mol Vis 12:441–444
Takada Y, Vijayasarathy C, Zeng Y, Kjellstrom S, Bush RA, Sieving PA (2008) Synaptic pathology in retinoschisis knockout (Rs1-/y) mouse retina and modification by rAAV-Rs1 gene delivery. Invest Ophthalmol Vis Sci 49:3677–3686

Tamayo ML, Bernal JE, Tamayo GE, Frias JL, Alvira G, Vergara O, Rodriguez V, Uribe JI, Silva JC (1991) Usher syndrome: results of a screening program in Colombia. Clin Genet 40:304–311

Tang Y, Wu S, Liu Q, Xie J, Zhang J, Han D, Lu Q, Lu Q (2015) Mertk deficiency affects macrophage directional migration via disruption of cytoskeletal organization. PLoS One 10(1): e0117787

Tantri A, Vrabec TR, Cu-Unjieng A, Frost A, Annesley WH Jr, Donoso LA (2004) X-linked retinoschisis: a clinical and molecular genetic review. Surv Ophthalmol 49:214–230

Thompson DA, Gyürüs P, Fleischer LL, Bingham EL, McHenry CL, Apfelstedt-Sylla E, Zrenner E, Lorenz B, Richards JE, Jacobson SG, Sieving PA, Gal A (2000) Genetics and phenotypes of RPE65 mutations in inherited retinal degeneration. Invest Ophthalmol Vis Sci 41(13):4293–4299

Thompson DA, Ali RR, Banin E, Branham KE, Flannery JG, Gamm DM, Hauswirth WW, Heckenlively JR, Iannaccone A, Jayasundera KT, Khan NW, Molday RS, Pennesi ME, Reh TA, Weleber RG, Zacks DN, Monaciano Consortium (2015) Advancing therapeutic strategies for inherited retinal degeneration: recommendations from the Monaciano Symposium. Invest Ophthalmol Vis Sci 56(2):918–931

Tolmachova T, Anders R, Abrink M, Bugeon L, Dallman MJ, Futter CE, Ramalho JS, Tonagel F, Tanimoto N, Seeliger MW, Huxley C, Seabra MC (2006) Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. J Clin Invest 116(2):386–394

Travis GH, Golczak M, Moise AR, Palczewski K (2007) Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. Annu Rev Pharmacol Toxicol 47:469–512

Tucker BA, Mullins RF, Stone EM (2014) Stem cells for investigation and treatment of inherited retinal disease. Hum Mol Genet 23(R1):R9–R16

van den Hurk JA, Hendriks W, van de Pol DJ, Oerlemans F, Jaissle G, Rüther K, Kohler K, Hartmann J, Zrenner E, van Bokhoven H, Wieringa B, Ropers HH, Cremers FP (1997) Mouse choroideremia gene mutation causes photoreceptor cell degeneration and is not transmitted through the female germline. Hum Mol Genet 6:851–858

Vasireddy V, Mills JA, Gaddameedi R, Basner-Tschakarjan E, Kohnke M, Black AD, Alexandrov K, Zhou S, Maguire AM, Chung DC, Mac H, Sullivan L, Gadue P, Bennicelli JL, French DL, Bennett J (2013) AAV-mediated gene therapy for choroideremia: preclinical studies in personalized models. PLoS One 8(5):e61396

Veleri S, Lazar CH, Chang B, Sieving PA, Banin E, Swaroop A (2015) Biology and therapy of inherited retinal degenerative disease: insights from mouse models. Dis Model Mech 8 (2):109–129

Vesk A, Nilsson SEG, Narfstrom K, Gal A (1999) Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. Genomics 57:57–61

Vollrath D, Feng W, Duncan JL, Yasumura D, D'Cruz PM, Chappelow A, Matthes MT, Kay MA, LaVail MM (2001) Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. Proc Natl Acad Sci U S A 98(22):12584–12589

Weleber RG, Francis PJ, Trzupek KM et al (2013) Leber congenital amaurosis. In: Pagon RA, Adam MP, Ardinger HH et al (eds) GeneReviews® [Internet]. University of Washington, Seattle, pp 1993–2016

Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH (1999) Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell 98(1):13–23

Wissinger B, Gamer D, Jägle H, Giorda R, Marx T, Mayer S, Tippmann S, Broghammer M, Jurklies B, Rosenberg T, Jacobson SG, Sener EC, Tatlipinar S, Hoyng CB, Castellan C, Bitoun P, Andreasson S, Rudolph G, Kellner U, Lorenz B, Wolff G, Verellen-Dumoulin C, Schwartz M, Cremers FP, Apfelstedt-Sylla E, Zrenner E, Salati R, Sharpe LT, Kohl S (2001) CNGA3 mutations in hereditary cone photoreceptor disorders. Am J Hum Genet 69 (4):722–737

Wu Z, Yang H, Colosi P (2010) Effect of genome size on AAV vector packaging. Mol Ther 18 (1):80–86

Yanoff M, Duker JS (2008) Ophthalmology, 3rd edn. Mosby, Edinburgh, pp 560–562

Zallocchi M, Binley K, Lad Y, Ellis S, Widdowson P, Iqball S, Scripps V, Kelleher M, Loader J, Miskin J, Peng Y-W, Wang W-M, Cheung L, Delimont D, Mitrophanous KA, Cosgrove D (2014) EIAV-based retinal gene therapy in the mouse model for Usher syndrome type 1B: development of UshStat. PLoS One 9(4):e94272

Zeng Y, Takada Y, Kjellstrom S, Hiriyanna K, Tanikawa A, Wawrousek E, Smaoui N, Caruso R, Bush RA, Sieving PA (2004) RS-1 gene delivery to an adult Rs1h knockout mouse model restores ERG b-wave with reversal of the electronegative waveform of X-linked retinoschisis. Invest Ophthalmol Vis Sci 45:3279–3285

Optic Nerve

Lynn K. Gordon

Contents

Abstract

Optic nerve diseases arise from many different etiologies including inflammatory, neoplastic, genetic, infectious, ischemic, and idiopathic. Understanding some of the characteristics of the most common optic neuropathies along with therapeutic approaches to these diseases is helpful in designing recommendations for individual patients. Although many optic neuropathies have no specific treatment, some do, and it is those potentially treatable or preventable conditions which need to be recognized in order to help patients regain their sight or develop a better understanding of their own prognosis. In this chapter several diseases are discussed including idiopathic intracranial

L.K. Gordon (✉)
Stein Eye Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
e-mail: lgordon@ucla.edu

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/164_2016_19

hypertension, optic neuritis, ischemic optic neuropathies, hereditary optic neuropathies, trauma, and primary tumors of the optic nerve. For each condition there is a presentation of the signs and symptoms of the disease, in some conditions the evaluation and diagnostic criteria are highlighted, and where possible, current therapy or past trials are discussed.

Keywords
Hereditary optic neuropathy • Idiopathic intracranial hypertension • Ischemia • Optic nerve • Optic neuritis • Trauma • Tumor

Abbreviations

IIH	Idiopathic intracranial hypertension
CSF	Cerebral spinal fluid
CVST	Cerebral venous sinus thrombosis
OCT	Optical coherence tomography
SLP	Scanning laser polarimetry
GCL+IPL	Ganglion cell layer and inner plexiform layers
NMO	Neuromyelitis optica
HIV	Human immunodeficiency viral infections
CDC	Centers for Disease Control
NAION	Non-arteritic, anterior ischemic optic neuropathy
GCA	Giant cell arteritis
DOA	Dominant optic atrophy
LHON	Leber hereditary optic neuropathy
CoQ10	Coenzyme Q10
TON	Traumatic optic neuropathy
ONSM	Optic nerve sheath meningiomas

1 Idiopathic Intracranial Hypertension

Idiopathic intracranial hypertension (IIH) was previously known as pseudotumor cerebri, a term which is no longer the preferred descriptor. IIH reflects a disease that is characterized by an elevation of intracranial pressure with a normal composition of cerebral spinal fluid (CSF) and without a structural lesion on neuroimaging (Friedman 2014; Friedman et al. 2013). Cerebral venous sinus thrombosis (CVST) can be a cause for elevated intracranial pressure and must be ruled out through use of specific imaging techniques such as magnetic resonance venography in patients with suspected IIH (Saposnik et al. 2011). Risk factors for CVST include coagulopathies, dehydration, and use of oral contraceptives (Saadatnia et al. 2009). Treatment of CVST includes the use of systemic anticoagulation or venous sinus

stenting, both of which have potential serious complications (Goodwin et al. 2014; Subramanian and Haq 2014).

The incidence of IIH is thought to be 1–3 per 100,000 people per year and is more common in females aged 20–40 (Friedman 2014). It has been associated with obesity, multiple medications, and certain endocrine abnormalities (Piper et al. 2015). The most common symptoms of IIH include headache in about 80–90%, transient visual obscurations in up to 72%, pulsatile tinnitus in 52%, and double vision in 23% (Wall et al. 2014). There may be palsies of cranial nerve six but otherwise the patient should have no other localizing neurologic signs or symptoms other than papilledema. Papilledema is present in the majority of patients but rarely can be unilateral or absent in IIH. Opening pressure measurements are obtained during a lumbar puncture to obtain CSF and must be done in proper position and without Valsalva in order to obtain an accurate and interpretable measurement (Avery 2014; Fridley et al. 2011; Friedman et al. 2013). Opening pressures in an adult should be above 250 mm H_2O and in a child should be above 280 mm H_2O in order to be confident in determining that the opening pressure is in fact elevated. The major long-term morbidity of IIH is vision loss, which can lead to irreversible optic atrophy.

Treatment of IIH includes lifestyle changes, medical management, and surgical management and was recently reviewed by Piper et al. (2015). In terms of lifestyle, there is some evidence to support the use of weight loss and diet in control of IIH. Acetazolamide is the most common medication used in the treatment of IIH and two randomized clinical trials, described below, demonstrated efficacy (Ball et al. 2011; Wall et al. 2014). However other medications, such as topiramate and Lasix, have also been used with success, but these have been reported in the absence of randomized clinical trials (Celebisoy et al. 2007; Finsterer et al. 2006). An open-label trial compared the use of acetazolamide to topiramate in patients with IIH; however, in contrast to other studies, the maximum dose used of acetazolamide in this study was relatively low at 1.5 g/day and topiramate was 150 mg/day (Celebisoy et al. 2007). In that study topiramate was judged to be as effective as acetazolamide for treatment of IIH.

Surgical therapy has included optic nerve sheath fenestration, lumboperitoneal shunting, ventriculoperitoneal shunting, and bariatric surgery for weight loss (Fridley et al. 2011; Goodwin et al. 2014; Lai et al. 2014; Levin et al. 2015; Menger et al. 2014). Again success with these therapies (Lee et al. 2005) has been reported in case series without the benefit of randomization. In addition to the potential complications at the time of surgical intervention, bariatric surgery may also lead to malabsorption and night vision problems secondary to vitamin A deficiency. Despite these potential risks, there has been a demonstrated benefit for select patients to undergo these procedures.

Despite an extensive literature on this disease and potential therapeutic interventions, there have been only two randomized prospective clinical trials, one done in the UK and published in 2011 and the other performed in the USA and published in 2014 (Ball et al. 2011; Wall et al. 2014). Both of these studies investigated the use of acetazolamide for the disease and also had an informal or

formal weight reduction plan for the subjects. The use of acetazolamide, up to 4 g/day, was associated with improvement in papilledema, decreased opening pressure of the CSF, improvement in vision quality of life studies, and a modest improvement in the mean deviation (MD) of the visual field; however, there was no benefit in control of headache (Wall et al. 2014). A limiting feature of US trial was that only individuals with mild visual field disturbances (−2 to −7 MD on the visual field) were included, and therefore the results cannot be extrapolated to individuals with more profound loss of vision.

2 Optic Neuritis

Optic neuritis is a term used for a group of inflammatory diseases that cause decreased optic nerve function (Galetta et al. 2015; Pau et al. 2011; Petzold and Plant 2014b; Shams and Plant 2009; Toosy et al. 2014). The optic disc may appear swollen in which case the term papillitis may be used. When the optic disc has a normal appearance, the term retrobulbar optic neuritis is used. Underlying causes of optic neuritis include demyelination, autoimmunity, or infection. Typical optic neuritis, the most common form of optic neuritis, is often associated with systemic demyelinating disease. Optic neuritis in the pediatric age group represents a special group of individuals which often requires therapeutic interventions (Collinge and Sprunger 2013). Atypical presentations of optic neuritis may include significant vision loss, absence of pain, bilateral and simultaneous vision loss, or a lack of significant improvement within 2–3 weeks of disease onset (Dumitrascu 2014). These deserve a comprehensive evaluation in an attempt to determine the underlying etiology.

2.1 Typical Optic Neuritis

Typical, demyelinating optic neuritis occurs most often in young adults and presents with a subacute, monocular involvement associated in up to 92% of cases with periorbital pain or pain on eye movement (Shams and Plant 2009). There is a female predilection and the vision loss generally occurs over a period of several days. Decreased color perception is a hallmark of optic neuritis and is often more profoundly affected than the actual visual acuity measurement. Spontaneous improvement to Snellen visual acuity >20/40 occurs in more than 90% of eyes. However deficits in contrast sensitivity may be permanent, and thinning of the nerve fiber layer is typically observed by optical coherence tomography (OCT) (Costello et al. 2011; Galetta et al. 2011; Lamirel et al. 2010; Sakai et al. 2011). We now understand that for future pharmacologic studies to detect benefits in treatment of optic neuritis, additional studies are required; retinal ganglion cell loss may be better investigated using new or improved techniques including OCT and scanning laser polarimetry (SLP) to evaluate the nerve fiber layer or the thickness of the ganglion cell layer and inner plexiform layers (GCL+IPL) (Bennett et al. 2015).

Other types of investigations commonly used in the clinic include low-contrast visual acuity and color vision, both of which may yield additional information about optic nerve function and complement both Snellen visual acuity and formal visual field measurements.

The definitive prospective, randomized clinical trial regarding corticosteroid therapy and optic neuritis was published more than 20 years ago (Beck et al. 1992; Gal et al. 2015). That trial demonstrated that, when initiated within the first 8 days of onset of optic neuritis, the use of intravenous methylprednisolone (1 g/day for 3 days) followed by an 11 day taper of oral prednisone both hastened recovery of vision and had an effect of decreasing another demyelinating event within the first 2 years but not thereafter. Studies that evaluated whether intravenous immunoglobulin (IVIg) therapy or plasma exchange was effective in the setting of typical optic neuritis did not reveal any benefit of therapy (Bennett et al. 2015). Erythropoietin, with or without prednisolone, demonstrated efficacy both in an experimental animal model and in a small series of human patients (Suhs et al. 2012). Whether there is a role for some of the newer medications used in treatment of systemic demyelinating diseases in the treatment of typical optic neuritis is not yet known, but there is some evidence for neuroprotection in animal models of the disease (Ledford 2015). Additional prospective clinical trials using multiple endpoints for evidence of efficacy in both preserving structure and function of the optic nerve will be helpful in identifying best treatment options (Qureshi et al. 2014).

2.2 Atypical, Autoimmune Optic Neuritis

Several types of atypical autoimmune optic neuritis have been described including neuromyelitis optica (NMO), single and relapsing autoimmune optic neuritis, chronic relapsing inflammatory optic neuritis, and acute disseminated encephalomyelitis (Jarius et al. 2014; Kleiter et al. 2015; Levin et al. 2013; Petzold and Plant 2014a, b). NMO is the most common form of the autoimmune optic neuritis syndromes and is the only one of these discussed further in this chapter.

Classic NMO was also called Devic's disease and is generally characterized by both optic neuritis and transverse myelitis (Kleiter et al. 2015; Wingerchuk et al. 2015). Although NMO can occur at any age, its onset in adults is generally around the fourth to fifth decade and is more common in females than males by a 3:1 ratio. In children NMO occurs in the first decade of life, usually around age 4. There is a racial and ethnic predilection and NMO has an increased incidence and prevalence in individuals from African, Asian, or Indian heritage.

The optic neuritis in NMO is typically bilateral and severe, demonstrates less improvement in visual recovery than observed in typical optic neuritis, and frequently recurs. The diagnosis of NMO requires two absolute criteria, optic neuritis and acute myelitis, and at least two of three additional criteria including (1) a negative brain MRI at disease onset, (2) spinal cord MRI with contiguous T2-weighted signal abnormality extending over three or more vertebral segments,

and (3) serologic testing that reveals the presence of circulating NMO-IgG antibodies. Patients who present with vision loss to the ophthalmologist may also have an NMO spectrum disease characterized by seropositivity for the NMO antibody against aquaporin 4 in association with optic neuritis but without spinal cord involvement (Wingerchuk et al. 2015).

Treatment for NMO during acute attacks include high-dose corticosteroids, plasma exchange, IVIg, and cyclophosphamide; however, no prospective clinical trials have been done in this disease, and therefore the treatment recommendations are based on retrospective clinical series or case reports (Papadopoulos et al. 2014; Yamasaki et al. 2016; Zephir et al. 2015). Other medications have been used chronically in NMO to prevent recurrences and include azathioprine, mycophenolate mofetil, methotrexate, and rituximab with reported varying success. Cyclosporine and mitoxantrone have also been used, but it is suggested due to concerns regarding toxicity that these be used in cases refractory to other agents (Papadopoulos et al. 2014).

2.3 Infectious Optic Neuritis

Infectious agents can cause optic neuritis or optic neuropathy and may cause either unilateral or bilateral vision loss (Bhai and Lyons 2015; Chi et al. 2012; de Mello Vitor et al. 2011; Goldsmith et al. 2000; Jaafar et al. 2012; Merkler et al. 2015). In some cases the onset is similar to optic neuritis but has atypical features such as a lack of pain, progression of vision loss over time, and the presence of a macular star, which is often seen in conjunction with optic disc swelling in patients with neuroretinitis or optic atrophy at the time of presentation. Optic neuritis, in particular in children, may also occur following a systemic viral or bacterial infection in which case it is termed a parainfectious optic neuritis and is believed to result from an immune mechanism not by direct infection. Infectious etiologies of optic nerve involvement include viruses, bacteria, and fungi. Patients with human immunodeficiency viral infections (HIV) may develop optic neuropathy secondary to the primary infection with HIV or secondary to a myriad of infectious agents, some of which rarely cause disease in immunocompetent individuals, a subject beyond the scope of this chapter. Three specific infections are further considered as symptomatic patients with these infections may present to the ophthalmologist: syphilis, Lyme disease, and cat-scratch disease or *Bartonella henselae* infection. Consultation with a specialist in infectious diseases is recommended in order to select the most current recommended treatment plan for any infectious disease that affects optic nerve function.

Syphilis is well known to the ophthalmologist through the old adage that it can do anything and present with involvement of any part of the eye (Bhai and Lyons 2015; Morshed and Singh 2015; Puech et al. 2010). Indeed, optic nerve involvement may be unilateral or bilateral and may present as optic perineuritis, optic neuritis, or papillitis. Central nervous system involvement may occur early, within weeks of onset of the disease or late, years, or decades following the initial

infection. Any ocular involvement deserves evaluation for neurosyphilis, and all patients with syphilis need to be evaluated for HIV. Serologic diagnosis for syphilis relies on either non-treponemal testing (Venereal Disease Research Laboratory [VDRL] or rapid plasma reagin [RPR]) or treponemal testing (fluorescent treponemal antibody absorbed [FTA-ABS], *T. pallidum* hemagglutination assay [TPHA], or *T. pallidum* particle agglutination assay [TPPA]) (Morshed and Singh 2015). The non-treponemal testing has a 1–2% false-positive rate in the USA and may be secondary to many different types of infections, autoimmune disease, intravenous drug abuse, or pregnancy. Treponemal testing may also have false-positive results from other spirochetal infections. An initial positive result on a non-treponemal test must be confirmed with a positive treponemal test. Following adequate therapy most, but not all, non-treponemal tests become negative; however, the specific treponemal tests remain positive. Diagnosis of neurosyphilis is particularly challenging as CSF VDRL can be negative in both immunocompetent and HIV-infected patients. In addition, up to 23% of patients with neurosyphilis may have a negative serum VDRL. In the appropriate setting, elevation of CSF white blood cells above 20 WBC/μL and increased CSF total protein may help confirm the suspicion of neurosyphilis. These patients should be evaluated and managed by an infectious disease specialist according to current Centers for Disease Control and Prevention (CDC) standards.

Optic nerve involvement in Lyme disease, or Lyme borreliosis, may include neuroretinitis, optic neuritis, ischemic optic neuropathy, or swelling of the optic disc (Bhatti 2007; Traisk and Lindquist 2012). Diagnosis may be challenging and relies on a combination of clinical involvement, risk of tick exposure, history of a classic rash, and either microbial or serologic evidence for disease. Lyme serology may be negative in early stages of disease; however, when neurologic involvement is present, serologic testing should be positive in the serum as well as the cerebrospinal fluid, which may also exhibit both pleocytosis and an elevated protein (Chi et al. 2012). If serologic testing is negative but suspicion is high for Lyme disease, retesting of convalescent serum, 2–6 weeks following presentation, may reveal positivity. Individuals in endemic areas may test positive by serology in the absence of clinical symptoms or disease, and therefore a positive serology must be interpreted in the context of other symptoms or signs. Additionally a two-step test is required for diagnosis; a positive ELISA serologic study is confirmed by a positive Western blot. Antimicrobial therapy is recommended in this disease, and the duration and route depend on disease manifestations.

Bartonella henselae is the organism associated with cat-scratch disease (CSD). Optic nerve involvement in CSD occurs at any age, but the mean patient age in a large series of patients was 27.8 years (Chi et al. 2012). Bilateral involvement may occur in up to 17% of affected individuals and initial visual acuity may range from 20/20 to count fingers (Chi et al. 2012). A history of cat scratch was present in about 67% of individuals in this study, and a macular star was observed in 45% of the patients. Diagnosis is made in part by serology for antibodies against the organism. Final visual outcome was equal to or better than 20/40 in about 68% of patients, and poor vision, equal to or less than 20/200, was observed in 5%; treatment did not

appear to be associated with recovery of visual function; however, this was a retrospective study (Chi et al. 2012). Treatment for this infection, which is typically a self-limiting disease, remains controversial. A review of all observational studies and randomized trials that looked at treatment for all *Bartonella* infections did not reveal any definitive benefit in terms of cure rate or time to resolution of disease (Prutsky et al. 2013).

3 Ischemic Optic Neuropathies

In patients older than the age of 50 years, ischemia is the most common cause of optic neuropathy with an estimated incidence of up to 10 per 100,000 individuals (Biousse and Newman 2015; Miller and Arnold 2015). The typical classification of ischemic optic neuropathy divides patients into two groups: non-arteritic and vasculitic. Of those with a non-arteritic ischemic optic neuropathy, the most common type is non-arteritic, anterior ischemic optic neuropathy or NAION. Although there are many types of vasculitis that can result in an ischemic optic neuropathy, the most common form is giant cell arteritis (GCA) and these are further discussed in this chapter (Singh et al. 2015; Weyand et al. 2012).

3.1 Non-arteritic Anterior Ischemic Optic Neuropathy (NAION)

NAION occurs in adults generally in their sixth and seventh decades of life and is more prevalent in Caucasians as compared to African-Americans or Hispanics (Miller and Arnold 2015). The typical presentation of a patient with NAION is an acute or subacute (hours to days) loss of vision which may be described as a defect in the visual field, clouding of their vision, or decreased brightness sensation. Although it is commonly considered, in contrast to optic neuritis, to be a painless disease, up to 12% of patients may note some discomfort around their eyes. Loss of vision may be progressive over days to weeks and final central visual acuity ranges from near normal to no light perception. Color vision loss is reported, but is generally concordant with loss of central vision, and visual field defects, although most frequently occur in an inferior and altitudinal distribution, may involve any portion of the field of vision.

Affected patients may have a history of diseases with vasculopathic consequences such as diabetes mellitus, hypertension, or hyperlipidemia, and increasingly there are associations between NAION and sleep apnea (Aptel et al. 2015; Archer and Pepin 2013; Li et al. 2007; Mojon et al. 2002; Palombi et al. 2006). Other risk factors that have been variably associated with NAION include nocturnal hypotension, severe anemia, hyperhomocysteinemia, coagulopathies, migraine, and smoking. NAION has also been observed during the perioperative period in intraocular surgery as well as in the earlier days of LASIK surgery when there was a significant elevation in intraocular pressure at the time of surgery (Cameron et al. 2001; Lee et al. 2000; Shetty et al. 2007).

Medications that have been associated with the development of NAION include interferon-α, erectile dysfunction drugs, and amiodarone, although the cause and effect relationship is not entirely convincing as the affected individuals may have confounding risk factors (Miller and Arnold 2015). The optic disc in the affected eye exhibits swelling, often with peripapillary hemorrhages, and the optic disc in the unaffected eye typically exhibits a crowded appearance with a small cup and has been called in the literature a "disc at risk."

A plethora of medical and surgical therapies, including hyperbaric oxygen, optic nerve sheath fenestration, optic neurotomy, diphenylhydantoin, aspirin, corticosteroids both systemic and intravitreal, anti-VEGF agents, and erythropoietin, have been used in NAION without convincing data for any therapeutic benefit (Miller and Arnold 2015). Evaluation and treatment for modifiable risk factors is perhaps the best care that a clinician can provide to their patient (Miller and Arnold 2015). The goal is to decrease the risk of fellow or second eye involvement in NAION, estimated to be around 15%, since bilateral NAION will likely produce significant morbidity and life changes to the patient. It is recommended that all patients undergo careful history and evaluation to detect use of medications that have been linked to NAION, to uncover medical risk factors for the disease, to diagnose sleep apnea, and to rule out vasculitis. Neuroimaging is not necessary in typical cases of NAION but should be performed in atypical cases. Similarly a hypercoagulable evaluation is generally not performed unless the patient is young or has a history that supports a hypercoagulable state. Low-dose aspirin has not been proven to be beneficial in protecting second eye involvement but is often prescribed for patients because of other vasculopathic risk factors in order to decrease incidence of stroke or myocardial infarction (Beck and Hayreh 2000).

3.2 Arteritic Ischemic Optic Neuropathy

The most common vasculitis of adults that results in ischemic optic neuropathy is GCA, also called temporal arteritis (Biousse and Newman 2015). The incidence of GCA varies from 1 to 20 cases per 100,000 persons older than 50 years and has a peak incidence in the eighth and ninth decades of life (Weyand and Goronzy 2014; Weyand et al. 2012). The ophthalmic presentation of GCA may be similar to NAION with sudden or subacute vision loss, but there are features that should raise the clinical suspicion of GCA. These features include transient vision loss or double vision preceding the acute or subacute event and systemic symptoms such as weight loss, fatigue, jaw or tongue claudication, new-onset headache, scalp pain or tenderness, and a history of polymyalgia rheumatica. Unfortunately up to 25% of affected individuals may have "occult" GCA without systemic signs or symptoms (Hayreh et al. 1998). Although typically the vision loss in GCA is more severe than in NAION, there is substantial overlap and this is not a good way to discriminate between the two diseases.

In contrast to NAION, clinical evaluation may reveal pale swelling of the optic disc in GCA; there may be involvement of concomitant retinal or choroidal

ischemia in GCA. Additionally, there is no association between GCA and a crowded optic disc or small cup to disc ratio. Palpation of the temporal arteries may reveal local tenderness or a ropey vasculature, in which case this area is a likely site for biopsy in order to obtain a higher probability for a positive result. Elevation of erythrocyte sedimentation rate, C-reactive protein, and platelet count, when present, is helpful in making the diagnosis of GCA, but the gold standard is a positive arterial biopsy with the presence of multinucleated giant cells and disruption of the internal elastic lamina. Although there has long been interest in identifying an infectious etiology for GCA, recent studies support a possible role for *varicella zoster*, yet the role for adjuvant antiviral therapy in this disease remains a question that should be answered through prospective trials (Mitchell and Font 2001; Nagel et al. 2013, 2015).

The goal of treatment of GCA is to prevent involvement of the second eye with the devastating consequence of bilateral vision loss. The mainstay of therapy includes high-dose glucocorticoid therapy, which has significantly reduced the incidence of visual complications (Chandran et al. 2015). In a recent study of visual complications in 204 patients with GCA, it was found that 1% of the patients in the series had complete and bilateral loss of vision, 3.4% of patients had complete monocular loss of vision, and 23% of patients had some type of visual signs or symptoms including blurred vision, double vision, loss of visual field, amaurosis fugax, and ventral retinal artery occlusion (Kermani et al. 2015; Singh et al. 2015). In this series those patients with visual manifestations were likely started on an average of 100 mg of oral prednisone in contrast to about 60 mg of oral prednisone for patients without visual symptoms. Many neuro-ophthalmologists would initiate high-dose pulse intravenous Solu-Medrol, at a dose of 1,000 mg of methylprednisolone per day for 3 days, as a starting corticosteroid for suspected GCA patients who present with vision loss; however, there are no evidence-based studies to support the use of intravenous over oral corticosteroids. Patients must be monitored for adverse effects of glucocorticoid therapy as they require long-term corticosteroid use with a slow taper over a period of many months. No definitive value for steroid-sparing agents has been demonstrated in the therapy of GCA.

4 Hereditary Optic Neuropathies

Hereditary optic neuropathies have an estimated prevalence of up to 1 per 10,000 individuals, and thus affected individuals will be seen in many practices of ophthalmology (Peragallo and Newman 2015; Pisano et al. 2015; Sabet-Peyman et al. 2012; Sadun et al. 2012; Sitarz et al. 2012; Yu-Wai-Man et al. 2011, 2014). In these patients there is selective loss of retinal ganglion cells, and the age of onset is variable but generally begins in the first several decades of life. The majority of cases occur either because of a mutation in the gene OPA1, which codes for a membrane protein of mitochondria, and this is termed dominant optic atrophy (DOA), or because of a point mutation in the mitochondrial DNA, called Leber hereditary optic neuropathy (LHON). Patients with either DOA or LHON may have

other neurologic involvement as well. Genetic counseling and formal evaluation is wise for any patient with a hereditary disease. In general, patients and at-risk relatives should be advised to avoid tobacco and heavy use of alcohol. Supportive care, referral to low-vision specialists, and psychological support are critically important for all patients with bilateral vision loss.

4.1 Leber Hereditary Optic Neuropathy (LHON)

Point mutations at three sites on complex 1 in the mitochondrial respiratory chain are the cause for LHON in more than 90% of cases. Disease penetrance is incomplete and about 50% of males and 10% of females will develop vision loss and optic atrophy. Asymptomatic carriers may have subclinical abnormalities on careful testing of color or contrast vision or visual physiologic testing using multifocal ERG or visual evoked potentials. They may also demonstrate some telangiectatic vessels near the optic disc or may be observed to have transient edema in the nerve fiber layer. Affected LHON patients exhibit an acute or subacute loss of vision in one eye with second eye involvement within several weeks and up to 1 year (Leruez et al. 2014; Newman 2012). Typically the papillomacular bundle is most affected. During the initial phases of visual symptoms, a variety of funduscopic abnormalities can be observed including retinal nerve fiber layer swelling, peripapillary telangiectasias, and optic disc hyperemia. Visual recovery is typically poor; however, patients with the 14484T>C mutation have some recovery in up to 58% of affected individuals. When recovery occurs, it generally is observed within the first year after disease onset, but it has been reported years following the disease onset in a small group of individuals (La Morgia et al. 2014; Lam et al. 2014).

Over the years many different types of therapies have been tried in patients with LHON. Vitamin supplements have been used without any evidence for efficacy. Brimonidine, a topical alpha-2-agonist that is widely used in glaucoma, showed some neuroprotective effects in animal models but failed to decrease second eye involvement in patients with LHON (Newman et al. 2005). Although there is great interest in the use of stem cell therapy in human disease, there is no evidence and no prospective clinical trial that demonstrates efficacy of this type of therapy in optic nerve diseases. There are several clinical trials listed as actively recruiting subjects on the clinicaltrials.gov Web site.

A ubiquinone analogue, coenzyme Q10 (CoQ10), has been used in many mitochondrial diseases in an attempt to bypass the complex 1, but there has not been evidence for efficacy in LHON. More potent analogues of ubiquinone, idebenone and EPI-743, have both been studied in patients with LHON with variable results. Idebenone at a dose of 900 mg/day was used for 24 weeks in a multicenter clinical trial of 85 patients and included individuals with up to a 5-year history of the disease (ClinicalTrials.gov identifier: NCT00747487) (Klopstock et al. 2011). The primary end point of visual acuity was not achieved in this study; however, there was a trend of visual improvement in the treated group, and if there were discordant visual acuities in the two eyes at the disease onset, then

there may have been a beneficial effect in the less affected eye (Klopstock et al. 2013). EPI-743 showed a benefit in a small trial of five patients (Sadun et al. 2012).

Gene therapy has been proposed for treatment of LHON and active trials are currently enrolling subjects (Feuer et al. 2015; Peragallo and Newman 2015; Yu-Wai-Man et al. 2014). Allotopic rescue is currently the modality being studied in the USA. In this model, the genetic material is inserted into the nucleus using a viral vector, and the new protein carries a targeting sequence that takes it to the mitochondria where it is able to replace the defective protein. This technique has been successful in an animal model of LHON and is now in human clinical trials. Safety of the vector in human patients was reported in 2015 (Feuer et al. 2015). Whether this or a different gene therapy approach will be successful awaits the completion of the investigations.

4.2 Dominant Optic Atrophy (DOA)

In DOA, a mutation is noted in the large gene that encodes for an inner mitochondrial membrane protein that is ubiquitously expressed. More than 200 different mutations have been described as disease associated. Vision loss typically begins in the first decade of life with variable final visual outcomes ranging from mild to profound vision loss. The disease is generally bilateral and symmetric with a slow progressive loss of vision. Visual evoked responses show both a delayed latency and diminished magnitude.

Idebenone was used at a dose of 270–675 mg/day in a small group of patients with OPA1 optic neuropathy with some evidence for benefit (Barboni et al. 2013). Additional trials are needed to be able to determine whether there is benefit for idebenone or EPI-743 in patients with DOA.

5 Traumatic Optic Neuropathies

Traumatic optic neuropathy (TON) can occur from a seemingly mild injury to the orbit or in association with major head injury. In a large retrospective study of individuals with head injuries, about 2.3% of patients had TON and of these cases about 10% were bilateral (Pirouzmand 2012; Steinsapir and Goldberg 2011). The presence of a nasoethmoid fracture was positively associated as a risk factor for TON. The three common causes include falls, traffic accidents, and assaults. The vast majority of affected individuals with TON, up to 70% of cases, occur in young men before the age of 40 years. Elderly are also at risk for TON because of the higher incidence of falls.

Treatment for TON remains controversial. One of the largest studies of patients with TON was published in 1999 and notably was a retrospective evaluation of the patients with their treatment and visual outcome (Levin et al. 1999). About 57% of individuals had spontaneous recovery as defined by improvement in best-corrected

Snellen visual acuity of at least three lines; 52% of individuals treated with corticosteroids and 32% of individuals who received surgery showed this degree of recovery. Many other studies have tried to examine the potential role for steroid therapy in TON, yet a comprehensive review of this topic reveals that there are no randomized prospective clinical trials that provide evidence for corticosteroids in improving final visual acuity (Yu-Wai-Man and Griffiths 2013).

There is recent interest in the use of other agents for neuroprotection in TON. Erythropoietin is one of these agents that showed experimental promise in bench science and in in vivo animal studies (Feuer et al. 2015). Two reports support a potential benefit for this therapy when delivered intravenously for three consecutive days in non-randomized trials (Kashkouli et al. 2011).

6 Tumors of the Optic Nerve

Optic nerve sheath meningiomas (ONSM) and optic gliomas are the two primary tumors of the optic nerve (Fried et al. 2013; Glass et al. 2014; Liu et al. 2013; Shapey et al. 2011; Shofty et al. 2014; Traber et al. 2015). ONSM typically present in the fourth–fifth decades of life and are more common in females; when they occur in children, they typically occur in the first decade of life and are most often associated with neurofibromatosis type 2 (Grob et al. 2015; Shapey et al. 2013). Patients often present with gradual, painless vision loss in association with decreased color perception. The optic disc may appear swollen or atrophic and optociliary shunt vessels may be observed at the disc. Neuroimaging reveals characteristic thickening of the optic nerve with a "tram track" appearance of preservation of the nerve itself with tumor involvement of the nerve sheath. Biopsy is not generally performed for diagnosis. Treatment and the timing of initiation of treatment are somewhat controversial. This is a slow-growing tumor and one cannot predict the clinical course of disease at the outset. Thus most neuro-ophthalmologists recommend careful initial follow-up every 3–6 months and neuroimaging every 6–12 months for the first several years in order to establish some evidence for the rate of progression. The decision to initiate specific treatment often occurs when progressive vision loss is observed or if the patient has a best-corrected visual acuity of 20/40 or worse. Radiation therapy is the treatment of choice, but there are differences in the route and total dose of therapy. More than 50% of treated patients will show some improvement in visual function; however, there may also be untoward complications of radiotherapy (Abouaf et al. 2012; Arvold et al. 2009; Brower et al. 2013; Pacelli et al. 2011; Paulsen et al. 2012; Vaphiades 2014). Surgical excision of tumor is reserved for disfiguring proptosis.

Optic pathway gliomas are usually seen in childhood, often in association with neurofibromatosis type 1 (Dodgshun et al. 2015; Liu et al. 2013). These tumors range in severity and can demonstrate spontaneous regression, stability, or progressive disease. Optic nerve gliomas are unusual tumors in adults and typically have a much more aggressive course (Traber et al. 2015). Gliomas in adults are often defined as a high-grade astrocytoma and generally have a progressive course

resulting in death within 1–2 years (Traber et al. 2015). The treatment of these aggressive tumors consists of radiotherapy with the addition of chemotherapy or anti-VEGF therapy, but the prognosis has not improved.

In the pediatric age group, there is significant controversy as to how to label these tumors, whether they are hamartomas or neoplasms (Liu et al. 2013). Arguments in favor of defining them as neoplasms include the fact that about half of these tumors are associated with vision loss and other symptoms such as obstructive hydrocephalus and endocrine abnormalities. Treatment of these tumors is offered when there is evidence for clinical or radiologic progression and typically consists of chemotherapy with an almost 70% progression-free 5 years. A worse prognosis is associated with young age, tumor involvement of the chiasm and hypothalamus, intraconal tumors.

7 Conclusions

This chapter reviewed a set of common diseases that affect the optic nerve function. For many diseases of the optic nerve itself, there are no evidence-based, prospective clinical trials to inform the treating physician about best care. However, some of the optic nerve diseases are treatable and responsive to therapy, and others appear not to have an effective therapeutic approach. New discoveries about pathophysiology of disease as well as novel therapeutic approaches are required in the future to prevent vision loss from optic nerve disease.

References

Abouaf L, Girard N, Lefort T et al (2012) Standard-fractionated radiotherapy for optic nerve sheath meningioma: visual outcome is predicted by mean eye dose. Int J Radiat Oncol Biol Phys 82:1268–1277

Aptel F, Khayi H, Pepin JL et al (2015) Association of nonarteritic ischemic optic neuropathy with obstructive sleep apnea syndrome: consequences for obstructive sleep apnea screening and treatment. JAMA Ophthalmol 133:797–804

Archer EL, Pepin S (2013) Obstructive sleep apnea and nonarteritic anterior ischemic optic neuropathy: evidence for an association. J Clin Sleep Med 9:613–618

Arvold ND, Lessell S, Bussiere M et al (2009) Visual outcome and tumor control after conformal radiotherapy for patients with optic nerve sheath meningioma. Int J Radiat Oncol Biol Phys 75:1166–1172

Avery RA (2014) Reference range of cerebrospinal fluid opening pressure in children: historical overview and current data. Neuropediatrics 45:206–211

Ball AK, Howman A, Wheatley K et al (2011) A randomised controlled trial of treatment for idiopathic intracranial hypertension. J Neurol 258:874–881

Barboni P, Valentino ML, La Morgia C et al (2013) Idebenone treatment in patients with OPA1-mutant dominant optic atrophy. Brain 136:e231

Beck RW, Hayreh SS (2000) Role of aspirin in reducing the frequency of second eye involvement in patients with non-arteritic anterior ischaemic optic neuropathy. Eye (Lond) 14(Pt 1):118. doi:10.1038/eye.2000.34

Beck RW, Cleary PA, Anderson MM Jr et al (1992) A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. The Optic Neuritis Study Group. N Engl J Med 326:581–588
Bennett JL, Nickerson M, Costello F et al (2015) Re-evaluating the treatment of acute optic neuritis. J Neurol Neurosurg Psychiatry 86:799–808
Bhai S, Lyons JL (2015) Neurosyphilis update: atypical is the new typical. Curr Infect Dis Rep 17:481
Bhatti MT (2007) Optic neuropathy from viruses and spirochetes. Int Ophthalmol Clin 47:37–66
Biousse V, Newman NJ (2015) Ischemic optic neuropathies. N Engl J Med 372:2428–2436
Brower JV, Amdur RJ, Kirwan J et al (2013) Radiation therapy for optic nerve sheath meningioma. Pract Radiat Oncol 3:223–228
Cameron BD, Saffra NA, Strominger MB (2001) Laser in situ keratomileusis-induced optic neuropathy. Ophthalmology 108:660–665
Celebisoy N, Gokcay F, Sirin H, Akyurekli O (2007) Treatment of idiopathic intracranial hypertension: topiramate vs acetazolamide, an open-label study. Acta Neurol Scand 116:322–327
Chandran A, Udayakumar PD, Kermani TA, et al (2015) Glucocorticoid usage in giant cell arteritis over six decades (1950 to 2009). Clin Exp Rheumatol 33:S-98–102
Chi SL, Stinnett S, Eggenberger E et al (2012) Clinical characteristics in 53 patients with cat scratch optic neuropathy. Ophthalmology 119:183–187
Collinge JE, Sprunger DT (2013) Update in pediatric optic neuritis. Curr Opin Ophthalmol 24:448–452
Costello FE, Klistorner A, Kardon R (2011) Optical coherence tomography in the diagnosis and management of optic neuritis and multiple sclerosis. Ophthalmic Surg Lasers Imaging 42 (Suppl):S28–S40
de Mello VB, Foureaux EC, Porto FB (2011) Herpes zoster optic neuritis. Int Ophthalmol 31:233–236
Dodgshun AJ, Elder JE, Hansford JR, Sullivan MJ (2015) Long-term visual outcome after chemotherapy for optic pathway glioma in children: site and age are strongly predictive. Cancer 121:4190–4196
Dumitrascu O, Gordon LK (2014) Atypical optic neuritis. AAO Focal Points 32:1–8
Feuer WJ, Schiffman JC, Davis JL, Porciatti V, Gonzalez P, Koilkonda RD, Yuan H, Lalwani A, Lam BL, Guy J (2015) Gene therapy for Leber hereditary optic neuropathy: initial results. Ophthalmology. doi:10.1016/j.ophtha.2015.10.025
Finsterer J, Foldy D, Fertl E (2006) Topiramate resolves headache from pseudotumor cerebri. J Pain Symptom Manage 32:401–402
Fridley J, Foroozan R, Sherman V et al (2011) Bariatric surgery for the treatment of idiopathic intracranial hypertension. J Neurosurg 114:34–39
Fried I, Tabori U, Tihan T et al (2013) Optic pathway gliomas: a review. CNS Oncol 2:143–159
Friedman DI (2014) The pseudotumor cerebri syndrome. Neurol Clin 32:363–396
Friedman DI, Liu GT, Digre KB (2013) Revised diagnostic criteria for the pseudotumor cerebri syndrome in adults and children. Neurology 81:1159–1165
Gal RL, Vedula SS, Beck R (2015) Corticosteroids for treating optic neuritis. Cochrane Database Syst Rev 8:CD001430
Galetta KM, Calabresi PA, Frohman EM, Balcer LJ (2011) Optical coherence tomography (OCT): imaging the visual pathway as a model for neurodegeneration. Neurotherapeutics 8:117–132
Galetta SL, Villoslada P, Levin N et al (2015) Acute optic neuritis: unmet clinical needs and model for new therapies. Neurol Neuroimmunol Neuroinflamm 2:e135
Glass LR, Canoll P, Lignelli A et al (2014) Optic nerve glioma: case series with review of clinical, radiologic, molecular, and histopathologic characteristics. Ophthal Plast Reconstr Surg 30:372–376

Goldsmith P, Jones RE, Ozuzu GE et al (2000) Optic neuropathy as the presenting feature of HIV infection: recovery of vision with highly active antiretroviral therapy. Br J Ophthalmol 84:551–553
Goodwin CR, Elder BD, Ward A et al (2014) Risk factors for failed transverse sinus stenting in pseudotumor cerebri patients. Clin Neurol Neurosurg 127:75–78
Grob SR, Jakobiec FA, Rashid A et al (2015) Pediatric optic nerve meningioma: diagnostic and therapeutic challenges. Ophthal Plast Reconstr Surg
Hayreh SS, Podhajsky PA, Zimmerman B (1998) Occult giant cell arteritis: ocular manifestations. Am J Ophthalmol 125:521–526
Jaafar J, Hitam WH, Noor RA (2012) Bilateral atypical optic neuritis associated with tuberculosis in an immunocompromised patient. Asian Pac J Trop Biomed 2:586–588
Jarius S, Wildemann B, Paul F (2014) Neuromyelitis optica: clinical features, immunopathogenesis and treatment. Clin Exp Immunol 176:149–164
Kashkouli MB, Pakdel F, Sanjari MS et al (2011) Erythropoietin: a novel treatment for traumatic optic neuropathy-a pilot study. Graefes Arch Clin Exp Ophthalmol 249:731–736
Kermani TA, Warrington KJ, Cuthbertson D et al (2015) Disease relapses among patients with giant cell arteritis: a prospective, longitudinal cohort study. J Rheumatol 42:1213–1217
Kleiter I, Gahlen A, Borisow N et al (2015) Neuromyelitis optica: evaluation of 871 attacks and 1,153 treatment courses. Ann Neurol. doi:10.1002/ana.24554
Klopstock T, Yu-Wai-Man P, Dimitriadis K et al (2011) A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. Brain 134:2677–2686
Klopstock T, Metz G, Yu-Wai-Man P et al (2013) Persistence of the treatment effect of idebenone in Leber's hereditary optic neuropathy. Brain 136:e230
La Morgia C, Carbonelli M, Barboni P et al (2014) Medical management of hereditary optic neuropathies. Front Neurol 5:141
Lai LT, Danesh-Meyer HV, Kaye AH (2014) Visual outcomes and headache following interventions for idiopathic intracranial hypertension. J Clin Neurosci 21:1670–1678
Lam BL, Feuer WJ, Schiffman JC et al (2014) Trial end points and natural history in patients with G11778A Leber hereditary optic neuropathy : preparation for gene therapy clinical trial. JAMA Ophthalmol 132:428–436
Lamirel C, Newman NJ, Biousse V (2010) Optical coherence tomography (OCT) in optic neuritis and multiple sclerosis. Rev Neurol (Paris) 166:978–986
Ledford H (2015) Drug that boosts nerve signals offers hope for multiple sclerosis. Nature 520:417
Lee AG, Kohnen T, Ebner R et al (2000) Optic neuropathy associated with laser in situ keratomileusis. J Cataract Refract Surg 26:1581–1584
Lee WB, Hamilton SM, Harris JP, Schwab IR (2005) Ocular complications of hypovitaminosis a after bariatric surgery. Ophthalmology 112:1031–1034
Leruez S, Amati-Bonneau P, Verny C et al (2014) Mitochondrial dysfunction affecting visual pathways. Rev Neurol (Paris) 170:344–354
Levin LA, Beck RW, Joseph MP et al (1999) The treatment of traumatic optic neuropathy: the International Optic Nerve Trauma Study. Ophthalmology 106:1268–1277
Levin MH, Bennett JL, Verkman AS (2013) Optic neuritis in neuromyelitis optica. Prog Retin Eye Res 36:159–171
Levin AA, Hess D, Hohler AD (2015) Treatment of idiopathic intracranial hypertension with gastric bypass surgery. Int J Neurosci 125:78–80
Li J, McGwin G Jr, Vaphiades MS, Owsley C (2007) Non-arteritic anterior ischaemic optic neuropathy and presumed sleep apnoea syndrome screened by the Sleep Apnea scale of the Sleep Disorders Questionnaire (SA-SDQ). Br J Ophthalmol 91:1524–1527
Liu GT, Katowitz JA, Rorke-Adams LB, Fisher MJ (2013) Optic pathway gliomas: neoplasms, not hamartomas. JAMA Ophthalmol 131:646–650
Menger RP, Connor DE Jr, Thakur JD et al (2014) A comparison of lumboperitoneal and ventriculoperitoneal shunting for idiopathic intracranial hypertension: an analysis of economic impact and complications using the Nationwide Inpatient Sample. Neurosurg Focus 37:E4

Merkler AE, Gaines N, Baradaran H et al (2015) Direct invasion of the optic nerves, chiasm, and tracts by Cryptococcus neoformans in an immunocompetent host. Neurohospitalist 5:217–222
Miller NR, Arnold AC (2015) Current concepts in the diagnosis, pathogenesis and management of nonarteritic anterior ischaemic optic neuropathy. Eye (Lond) 29:65–79
Mitchell BM, Font RL (2001) Detection of varicella zoster virus DNA in some patients with giant cell arteritis. Invest Ophthalmol Vis Sci 42:2572–2577
Mojon DS, Hedges TR 3rd, Ehrenberg B et al (2002) Association between sleep apnea syndrome and nonarteritic anterior ischemic optic neuropathy. Arch Ophthalmol 120:601–605
Morshed MG, Singh AE (2015) Recent trends in the serologic diagnosis of syphilis. Clin Vaccine Immunol 22:137–147
Nagel MA, Khmeleva N, Boyer PJ, Choe A, Bert R, Gilden D (2013) Varicella zoster virus in the temporal artery of a patient with giant cell arteritis. J Neurol Sci 335:228–230
Nagel MA, White T, Khmeleva N et al (2015) Analysis of varicella-zoster virus in temporal arteries biopsy positive and negative for giant cell arteritis. JAMA Neurol 72:1281–1287
Newman NJ (2012) Treatment of hereditary optic neuropathies. Nat Rev Neurol 8:545–556
Newman NJ, Biousse V, David R et al (2005) Prophylaxis for second eye involvement in Leber hereditary optic neuropathy: an open-labeled, nonrandomized multicenter trial of topical brimonidine purite. Am J Ophthalmol 140:407–415
Pacelli R, Cella L, Conson M et al (2011) Fractionated stereotactic radiation therapy for orbital optic nerve sheath meningioma – a single institution experience and a short review of the literature. J Radiat Res 52:82–87
Palombi K, Renard E, Levy P et al (2006) Non-arteritic anterior ischaemic optic neuropathy is nearly systematically associated with obstructive sleep apnoea. Br J Ophthalmol 90:879–882
Papadopoulos MC, Bennett JL, Verkman AS (2014) Treatment of neuromyelitis optica: state-of-the-art and emerging therapies. Nat Rev Neurol 10:493–506
Pau D, Al Zubidi N, Yalamanchili S, Plant GT, Lee AG (2011) Optic neuritis. Eye (Lond) 25:833–842
Paulsen F, Doerr S, Wilhelm H et al (2012) Fractionated stereotactic radiotherapy in patients with optic nerve sheath meningioma. Int J Radiat Oncol Biol Phys 82:773–778
Peragallo JH, Newman NJ (2015) Is there treatment for Leber hereditary optic neuropathy? Curr Opin Ophthalmol 26:450–457
Petzold A, Plant GT (2014a) Chronic relapsing inflammatory optic neuropathy: a systematic review of 122 cases reported. J Neurol 261:17–26
Petzold A, Plant GT (2014b) Diagnosis and classification of autoimmune optic neuropathy. Autoimmun Rev 13:539–545
Piper RJ, Kalyvas AV, Young AM et al (2015) Interventions for idiopathic intracranial hypertension. Cochrane Database Syst Rev 8:CD003434
Pirouzmand F (2012) Epidemiological trends of traumatic optic nerve injuries in the largest Canadian adult trauma center. J Craniofac Surg 23:516–520
Pisano A, Preziuso C, Iommarini L et al (2015) Targeting estrogen receptor beta as preventive therapeutic strategy for Leber's hereditary optic neuropathy. Hum Mol Genet 24:6921–6931
Prutsky G, Domecq JP, Mori L et al (2013) Treatment outcomes of human bartonellosis: a systematic review and meta-analysis. Int J Infect Dis 17:e811–e819
Puech C, Gennai S, Pavese P et al (2010) Ocular manifestations of syphilis: recent cases over a 2.5-year period. Graefes Arch Clin Exp Ophthalmol 248:1623–1629
Qureshi SS, Beh SC, Frohman TC, Frohman EM (2014) An update on neuro-ophthalmology of multiple sclerosis: the visual system as a model to study multiple sclerosis. Curr Opin Neurol 27:300–308
Saadatnia M, Fatehi F, Basiri K et al (2009) Cerebral venous sinus thrombosis risk factors. Int J Stroke 4:111–123
Sabet-Peyman EJ, Khaderi KR, Sadun AA (2012) Is Leber hereditary optic neuropathy treatable? Encouraging results with idebenone in both prospective and retrospective trials and an illustrative case. J Neuroophthalmol 32:54–57

Sadun AA, Chicani CF, Ross-Cisneros FN et al (2012) Effect of EPI-743 on the clinical course of the mitochondrial disease Leber hereditary optic neuropathy. Arch Neurol 69:331–338
Sakai RE, Feller DJ, Galetta KM et al (2011) Vision in multiple sclerosis: the story, structure-function correlations, and models for neuroprotection. J Neuroophthalmol 31:362–373
Saposnik G, Barinagarrementeria F, Brown RD Jr et al (2011) Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 42:1158–1192
Shams PN, Plant GT (2009) Optic neuritis: a review. Int MS J 16:82–89
Shapey J, Danesh-Meyer HV, Kaye AH (2011) Diagnosis and management of optic nerve glioma. J Clin Neurosci 18:1585–1591
Shapey J, Sabin HI, Danesh-Meyer HV, Kaye AH (2013) Diagnosis and management of optic nerve sheath meningiomas. J Clin Neurosci 20:1045–1056
Shetty R, Babu RB, Suresh M et al (2007) Neuro-ophthalmic disorders presenting as a diagnostic surprise during pre-LASIK evaluation. J Cataract Refract Surg 33:1653–1656
Shofty B, Constantini S, Bokstein F et al (2014) Optic pathway gliomas in adults. Neurosurgery 74:273–279, discussion 279-80
Singh AG, Kermani TA, Crowson CS et al (2015) Visual manifestations in giant cell arteritis: trend over 5 decades in a population-based cohort. J Rheumatol 42:309–315
Sitarz KS, Chinnery PF, Yu-Wai-Man P (2012) Disorders of the optic nerve in mitochondrial cytopathies: new ideas on pathogenesis and therapeutic targets. Curr Neurol Neurosci Rep 12:308–317
Steinsapir KD, Goldberg RA (2011) Traumatic optic neuropathy: an evolving understanding. Am J Ophthalmol 151:928–933.e2
Subramanian PS, Haq A (2014) Cerebral venous sinus thrombosis and stenosis in pseudotumor cerebri syndrome. Int Ophthalmol Clin 54:61–71
Suhs KW, Hein K, Sattler MB et al (2012) A randomized, double-blind, phase 2 study of erythropoietin in optic neuritis. Ann Neurol 72:199–210
Toosy AT, Mason DF, Miller DH (2014) Optic neuritis. Lancet Neurol 13:83–99
Traber GL, Pangalu A, Neumann M et al (2015) Malignant optic glioma – the spectrum of disease in a case series. Graefes Arch Clin Exp Ophthalmol 253:1187–1194
Traisk F, Lindquist L (2012) Optic nerve involvement in Lyme disease. Curr Opin Ophthalmol 23:485–490
Vaphiades M (2014) Radiation optic neuropathy after proton beam therapy for optic nerve sheath meningioma. J Neuroophthalmol 34:101–102
Wall M, McDermott MP, Kieburtz KD et al (2014) Effect of acetazolamide on visual function in patients with idiopathic intracranial hypertension and mild visual loss: the idiopathic intracranial hypertension treatment trial. JAMA 311:1641–1651
Weyand CM, Goronzy JJ (2014) Clinical practice. Giant-cell arteritis and polymyalgia rheumatica. N Engl J Med 371:50–57
Weyand CM, Liao YJ, Goronzy JJ (2012) The immunopathology of giant cell arteritis: diagnostic and therapeutic implications. J Neuroophthalmol 32:259–265
Wingerchuk DM, Banwell B, Bennett JL et al (2015) International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 85:177–189
Yamasaki R, Matsushita T, Fukazawa T et al (2016) Efficacy of intravenous methylprednisolone pulse therapy in patients with multiple sclerosis and neuromyelitis optica. Mult Scler 22: 1337–1348
Yu-Wai-Man P, Griffiths PG (2013) Steroids for traumatic optic neuropathy. Cochrane Database Syst Rev 6:CD006032
Yu-Wai-Man P, Griffiths PG, Chinnery PF (2011) Mitochondrial optic neuropathies – disease mechanisms and therapeutic strategies. Prog Retin Eye Res 30:81–114
Yu-Wai-Man P, Votruba M, Moore AT, Chinnery PF (2014) Treatment strategies for inherited optic neuropathies: past, present and future. Eye (Lond) 28:521–537
Zephir H, Bernard-Valnet R, Lebrun C et al (2015) Rituximab as first-line therapy in neuromyelitis optica: efficiency and tolerability. J Neurol 262:2329–2335

Index

S.M. Whitcup, D.T. Azar (eds.), *Pharmacologic Therapy of Ocular Disease*,
Handbook of Experimental Pharmacology 242, DOI 10.1007/978-3-319-58290-0