Title
Influence of plasma protein binding on pharmacokinetics and pharmacodynamics of drugs

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Publication Date
1982

Peer reviewed|Thesis/dissertation

INFLUENCE OF PLASMA PROTEIN BINDING ON PHARMACOKINETICS AND PHARMACODYNAMICS OF DRUGS

by

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B.S. in Pharmacy
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1977

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

Acknowledgement

With the deepest gratitude, I wish to thank Dr. Svein Øie, for his unselfishness in sharing his wisdom, inspiration, unbounded support, encouragement, respectful tolerance, and his friendship.

My most sincere and grateful thanks also go to Dr. Thomas N. Tozer, Dr. Leslie Z. Benet, Dr. Kathleen M. Giacomini, Ms. Barbara Woodruff, Ms. Rebekah Levy, and Mr. Frank Fiori, for their generous time, energy, valuable suggestions, kind instruction, considerate friendship, and their help.

Last, but not least, I thank my wife Woan-ching, not only for her infinite help at home, but also for her painstaking assistance in the laboratory.

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Abstract

The influence of altered serum protein binding on the disposition of disopyramide and the pharmacological response to the drug (ΔQRS duration) was studied in the rabbit. Binding changes of disopyramide were achieved by injection of human glycoprotein fraction VI.

The total concentration-response relationship of RS-disopyramide was found to be different between the glycoprotein-treated rabbits and control rabbits. The unbound concentration-response relationship, on the other hand, was the same.

During constant infusion of R-disopyramide, the glycoprotein injection increased the total drug concentration in serum at steady state while the unbound concentration was unaltered. The glycoprotein injection, however, decreased the unbound concentration and increased the total concentration during the steady-state infusion of S-disopyramide. In both experiments, the ΔQRS duration reflected the change in the unbound drug concentration and not in the total concentration.

For a drug with concentration-dependent serum protein binding, the unbound fraction of drug decreases during the drug elimination process. The clearance of the drug at a given blood flow is lower than would be expected from the observed unbound fraction in venous blood from a noneliminating organ. Simulations showed that consideration of concentration-dependent binding during drug elimination process is important when the intrinsic clearance is higher than the blood flow and when the unbound drug concentration is much greater than the dissociation equilibrium constant of the binding complex.

The effect of serum protein binding on the clearance of a medium-to-high extraction ratio drug, S-disopyramide, was studied in individual

rabbits by successive injections of increasing doses of human glycoprotein. The results are consistent with predictions based on both the "well-stirred" and "parallel tube" models. However, the variation was too large to determine accurately the clearance-drug binding relationship.

Definitions and terminology

unbound volume of distribution. Volume of distribution of drugs based on unbound drug concentration in serum; the total amount of drug in the body divided by drug concentration in serum water.

total volume of distribution. Volume of distribution of drugs based on total drug concentration in serum; the total amount of drug divided by total drug concentration in serum.

unbound clearance. Drug clearance based on unbound drug concentration in serum; the rate of elimination of drug in the body or in a specific organ divided by drug concentration in serum water.

total clearance. Drug clearance based on total drug concentration in serum; the rate of drug elimination divided by total drug concentration in serum.

blood clearance. Drug clearance based on total drug concentration in blood; the rate of drug elimination divided by total drug concentration in blood.

unbound fraction. Fraction of drug in serum that is not bound to serum protein; drug concentration in serum water divided by total drug concentration in serum.

unbound fraction in blood. Fraction of drug in blood that is not bound to serum protein or blood cells; drug concentration in serum water divided by total drug concentration in blood.

extraction ratio. The fraction of drug eliminated by the eliminating organ during a single passage through the organ; it is equal to the ratio of blood clearance of the eliminating organ divided by blood flow.

availability. The fraction of drug which is not eliminated by the eliminating organ after a single passage through the organ; it is equal to one minus the extraction ratio.

Introduction

The focus of this research is the study of the role of serum protein binding on pharmacokinetics and pharmacodynamics of drugs. Many models, hypotheses, and assumptions postulating the role of serum protein binding in drug disposition and drug action have evolved over the years; however, they have not been substantially documented. The influence of serum protein binding on the disposition of disopyramide and the pharmacological response in the rabbit were, therefore, studied as a model.

The first chapter reviews the current literature and necessary background for this research; it includes proposed theories on the influence of serum protein binding on drug disposition and pharmacological response, factors causing variation in serum protein binding, and the properties of the model drug, disopyramide. The procedures and materials which were generally used in this research are presented in Chapter II. The observed electrocardiographic and pharmacokinetic properties of R- and S-disopyramide that dictated the experimental design in Chapters V and VII are presented in Chapter III.

The first objective of this research is to identify whether the unbound drug concentration or the total drug concentration in serum reflects the pharmacological response to drugs when serum protein binding is altered. To meet the objective, two studies were carried out to test the influence of inter- and intrasubject

differences in serum protein binding on the pharmacological response. These studies are described in Chapter IV and Chapter IV, respectively.

The second objective of this research is to study the influence of serum protein binding on the disposition of drugs. The influence of altered serum protein binding on the clearance of S-disopyramide in the rabbit was therefore studied and is presented in Chapter VII. A theoretical derivation, simulation, and discussion is presented in Chapter VI, which focuses on the influence of concentration-dependent serum protein binding on the hepatic elimination of drugs.

CHAPTER I.

BACKGROUND

The background of the research is reviewed in this chapter. The first two sections present the current concepts regarding the influence of protein binding on pharmacokinetics and pharmacodynamics and examine the rationale associated with the acceptance of these concepts.

Because of the inter- and intrasubject variation in serum protein binding, the study of the influence of serum protein binding on drug disposition and pharmacological response is not only scientifically interesting, but clinically important. In addition to the intrinsic sources of variation, such as heredity, age, and gender, serum protein binding is altered by disease states and various physiological conditions. Furthermore, endogenous and exogenous compounds may also alter serum protein binding of drugs by displacement. These variations in serum protein binding and possible mechanisms causing the variations are discussed in Section I-4. Background information about various binding proteins, as well as, their role in drug binding are presented in Section I-3.

Disopyramide was used as the model drug in this study, mainly because its binding in rabbit can be altered and its pharmacological response and drug concentration can be quantitated. The physico-chemical properties, pharmacodynamic properties, pharmacokinetic properties, and clinical use and toxicity of disopyramide are reviewed in Section I-5.

I-1. Protein Binding and Drug Disposition

An alteration in serum protein binding often causes changes in drug concentration as well as pharmacokinetic parameters. Two independent pharmacokinetc parameters, the volume of distribution and clearance, have been related to the extent of serum protein binding of drugs.

I-1-a. Protein Binding and Volume of Distribution of Drugs

Volume of distribution of a drug in terms of total serum concentration has been shown to be a function of unbound fraction in serum based on mass balance considerations. Gillette (135) showed that the volume of distribution, V, can be expressed by:

$$V = \alpha \cdot (V_f + X \cdot V_T) + (1 - \alpha) \cdot V_p \qquad \text{(Eq. I-1)}$$

where α is the fraction unbound in serum, V_f is the volume into which the unbound drug is distributed, X is the ratio of tissue drug concentration to unbound drug concentration, V_T is the tissue volume, and V_p is the apparent volume of distribution of the serum proteins to which the drug binds.

A simplified relationship, based on the physiological concepts of Gillette (132,133,135,136) was proposed as follows (130,408):

$$V = V_p + \frac{\alpha}{\alpha_T} \cdot V_T \qquad \text{(Eq. I-2)}$$

where V_p is the serum volume, V_T is the volume outside serum

into which the drug distributes, and α and α_T are the fractions unbound in these two components.

The relationship described by Eq. I-2 does not take into account that serum proteins are distributed throughout the extracellular fluids. When conditions alter the binding to proteins in serum such as binding displacement by other ligands, similar changes can be expected in the binding to these proteins located in other extracellular fluids. In other words, α_T will also change when α is altered. The difficulty of having two interrelated variables also exists in the approach of Eq. I-1, in which V_p is defined as the apparent volume of distribution of the serum proteins rather than the physical volume of serum. An alteration in binding protein concentration in serum which changes the unbound fraction of drugs, α, will also change the apparent volume of distribution of binding proteins, V_p, unless the alteration in amount of binding protein in the body happens to parallel changes in serum protein concentration.

Øie and Tozer (283) have proposed another relationship as follows:

$$V = V_p \cdot (1 + R_{E/I}) + \alpha \cdot V_p \cdot (V_E/V_p - R_{E/I}) + \alpha \cdot \frac{V_R}{\alpha_R} \qquad \text{(Eq. I-3)}$$

where V_p is the serum volume, V_E is the extracellular space minus the serum volume, and V_R is the physical volume into which the drug distributes minus extracellular space, $R_{E/I}$ is the

ratio of total number of binding sites or the amount of serum proteins in extracellular fluids outside the serum to that in serum, and α_R is the unbound drug fraction outside the extracellular space.

This relationship is similar to that proposed by Gillette (135; Eq. I-1) but includes terms for the intravascular-extravascular distribution of the binding protein as well as the actual volumes of the these extracellular fluids. Similarly in Eq. I-1, under the circumstance that the binding changes are due to the alteration in serum protein concentration, $R_{E/I}$ might also change in addition to the unbound fraction change.

Equations I-2 and I-3 can be simplified as follows when the volume of distribution is high relative to V_p or $V_p \cdot (1 + R_{E/I})$ terms in the equations:

$$V = V_T \cdot \frac{\alpha}{\alpha_T} \qquad \text{(Eq. I-4-a)}$$

or

$$V = V_R \cdot \frac{\alpha}{\alpha_R} \quad . \qquad \text{(Eq. I-4-b)}$$

These relationships predict that the volume of distribution based on total serum concentration will be proportional to the unbound fraction of drug in serum as has been shown for drugs like propranolol (49,109) and quinidine (118,144).

Equation I-3 is particularly useful for drugs with a low apparent volume of distribution (*i.e.* <0.2 ℓ/kg for albumin bound drug) in analyzing and predicting alterations in the

volume of distribution when there is an alteration in unbound fraction in serum. The alteration of the volume of distribution of tolbutamide in acute viral hepatitis patients is an example of this (283,404). It is also intersting to note that when a drug is only distributed to the extracellular fluids and cannot enter the cells, the minimum value for the apparent volume of distribution will be $V_p \cdot (1 + R_{E/I})$ no matter how tightly the drug is bound. In other words, the apparent volume of distribution based on serum drug concentration may be a constant which is independent of unbound drug fraction at the extreme condition.

Gillette (134) also showed that the unbound volume of distribution can be related to the concentration of binding sites and the affinity of the binding sites as follows:

$$V_{df} = V_f + \frac{Vab \cdot Bta \cdot Ka}{1 + Ka \cdot Cf} + \frac{Vbb \cdot Btb \cdot Kb}{1 + Kb \cdot Cf} \qquad \text{(Eq. I-5)}$$

where: V_{df} is the apparent volume of distribution of unbound drug; V_f is the physical volume to which the unbound drug distributes as defined in Eq. I-1; Cf is the unbound drug concentration in the body; Vab, Bta, Ka are the distribution volume of albumin, the serum albumin concentration, and the affinity constant of the drug to albumin, respectively; and Vbb, Btb, Kb are the distribution volume, total concentration, and affinity of tissue binding sites.

Although the parameters in Eq. I-5 may not be readily measurable, this relationship, however, reveals some clinically

important aspects of drug disposition. It states that for an albumin bound drug, the unbound volume of distribution will not be altered by differences in serum albumin binding as long as the total amount of albumin remains constant. The number of binding sites and physical volume determine the unbound volume of distribution.

Considering the partitioning of drug to lipophilic tissues and multiple binding sites in serum and in tissues, Eq. 1-5 can be expanded into a general form. The unbound volume of distribution, Vu, is equal to:

$$Vu = Vw + \Sigma \lambda_i \cdot V_{Li} + \Sigma \frac{n_i \cdot Ap_i}{Kd_i + Cu} \qquad \text{(Eq. 1-6)}$$

where Vw is the volume of body water into which the drug distributes--it can be plasma water, extracellular water, or total water, depending on the accessibility of various tissues for drug; Cu is the unbound drug concentration, which is assumed to be the same throughout the distribution space; λ_i is the partition coefficient for the individual lipophilic tissue i; V_{Li} is the physical volume of the lipophilic tissue; Kd_i is the dissociation constant of the individual binding sites; and Ap_i is the total amount of binding sites of each individual class, and each class has n_i independent sites. For a drug with linear binding in both serum and tissue, Vu can be approximated as:

$$Vu = Vw + \Sigma \lambda_i \cdot V_{Li} + \Sigma n_i \cdot Ap_i / Kd_i \quad . \qquad \text{(Eq. 1-7)}$$

In other words, unbound volume of distribution is a function

of the body water to which the drug distributes, the partition coefficient to lipophilic tissues, their volume (size), the number of binding sites and their dissociation constants. The distribution of binding macromolecules in various tissues or serum will, therefore, not affect the unbound volume of distribution as it will the total apparent volume of distribution, unless the binding affinity differs significantly in different body fluids.

For a very lipophilic drug, the partition in lipophilic tissue will be the dominant factor in drug distribution, and the $\Sigma\, n_i \cdot Ap_i / (Kd_i + Cu)$ term is negligible in Eq. I-6. The unbound volume of distribution will be a constant independent of the alteration in drug binding in serum or elsewhere, as well as of the drug concentration. The volume of distribution of total serum drug is, on the other hand, proportional to unbound fraction of drug in serum.

For a lipophobic drug, the term $\Sigma\, n_i \cdot Ap_i/(Kd_i + Cu)$ in Eq. I-6 dominates and apparent volume of distribution of unbound drug depends on the extent of drug binding. The stronger the binding, the larger is the unbound volume of distribution. The volume of distribution with respect to total drug concentration in serum, on the other hand, will become a complicated function of drug binding in serum and elsewhere and the distribution of binding proteins.

As shown in Eq. I-6, unbound volume of distribution is

concentration-dependent provided Cu is of similar magnitude or higher than the value of Kd_i and the concentration-dependent term is not negligible in comparison to all other terms. The concentration-dependent unbound volume of distribution is of course much more easily demonstrated for drugs with low lipophilic partitioning than drugs with high lipophilic partitioning.

I-1-b. Protein Binding and Clearance of Drugs

The influence of serum protein binding on drug clearance has focused primarily on drugs which are mainly eliminated *via* the hepatic route. A statistically significant correlation between clearance and unbound fraction of drug in plasma has been shown for a number of substances having a low hepatic extraction ratio like bilirubin (279), dicumarol (222), sulfisoxazole (417), and warfarin (228). For drugs that are highly hepatically extracted, like quinidine in the rabbit (144), the hepatic clearance is close to blood flow and independent of the unbound fraction, and the unbound clearance is inversely proportional to unbound fraction in blood. The observed discrepancy has been well explained by the concept of perfusion-limited drug elimination (288,408).

Based on a "well-stirred" model for perfusion of the liver (327), Wilkinson and Shand (408) presented an equation that related hepatic clearance (Cl_{HB}) to the hepatic blood flow (Q) and unbound fraction of drug in blood (α_B):

$$Cl_{HB} = \frac{Q \cdot \alpha_B \cdot Cl_I}{Q + \alpha_B \cdot Cl_I} \qquad \text{(Eq. I-8)}$$

where Cl_I is the intrinsic clearance, which is the rate of hepatic drug elimination divided by the unbound drug concentration in the sinusoid. The basic assumption of the model is that the drug concentration leaving the liver is the same as the drug concentration inside the sinusoid, and that unbound drug in the hepatocyte is instantaneously in equilibrium with the unbound drug in the sinusoid.

When the value of $\alpha_B \cdot Cl_I$ is much larger than that of Q, Eq. I-8 can be approximated as:

$$Cl_{HB} = Q \qquad \text{(Eq. I-9)}$$

$$Cl_u = Q / \alpha_B \qquad \text{(Eq. I-10)}$$

where Cl_u is the unbound clearance. When the value of $\alpha_B \cdot Cl_I$ is much smaller than that of Q, Eq. I-8 can be approximated as:

$$Cl_{HB} = \alpha_B \cdot Cl_I \qquad \text{(Eq. I-11)}$$

$$Cl_u = Cl_I \qquad \text{(Eq. I-12)}$$

These approximations are consistent with empirical observations (144, 222,228,279,417), and have been used extensively to predict and explain pharmacokinetic data.

As an alternative approach, if one were to assume the sinusoids as "parallel tubes" and that the concentration of drug inside the sinusoids declined exponentially along the direction of flow because of continuous

elimination by hepatocytes with the same intrinsic clearance (194,409-411), this would result in another relationship with the same parameters as the "well-stirred" model (288):

$$Cl_{HB} = Q \cdot [1 - exp\ (\frac{-\alpha_B \cdot Cl_I}{Q})] \quad . \qquad \text{(Eq. I-13)}$$

At extreme conditions, where the value of $\alpha_B \cdot Cl_I$ is either much larger or much smaller than that of Q, the equation can be simplified to the same relationship shown in Eqs. I-9 to -12 and can be used to analyze or predict clearance changes as well as the "well-stirred" model, when any of the parameters, α_B, Cl_I or Q is altered.

Although the two models are mathematically similar at the extreme conditions, there is a discrepancy in clearance prediction when blood flow or protein binding is altered (288). The greatest difference between the models was demonstrated in the predicted change of the availability ($1 - Cl_{HB} / Q$) with alteration in either hepatic blood flow or unbound fraction for drugs with high values of the extraction ratio (Cl_{HB} / Q). The availability changes linearly with blood flow for the "well-stirred" model and exponentially for the "parallel tube" model; whereas the availability varies in inverse proportion to the unbound fraction in the "well-stirred" model and varies exponentially with the unbound fraction in the "parallel tube" model. Based on this discrepancy, Pang and Rowland (289,290) changed the blood flow in the perfused rat liver and concluded that the "well-stirred" model is a better model for lidocaine in the rat. Keiding and Chiarantini (195), on the other hand, showed that the "parallel tube" model better describes the clearance of

galactose in the perfused rat liver when blood flow is altered.

The influence of protein binding on hepatic clearance is more complicated if the drug-protein complex dissociation is rate-limiting. Jansen (175) considered the potential influence of protein binding kinetics in both the "well-stirred" and the "parallel tube" models and showed that a significant decrease in the extraction ratio might result if drug-protein complex dissociation is slow for drugs with an unbound fraction of 0.01 or less.

Further complication of the relationship occurs if the diffusion of drug from the sinusoid to the hepatocyte is not "instantaneous". For example, Gillette and Pang (136) considered the potential influence of drug diffusion from sinusoid to hepatocyte as a rate-limiting step and proposed a relationship incorporating the diffusion constant (Q_{dif}) into an equation based on the "well-stirred" model:

$$Cl_{HB} = \frac{\alpha_B \cdot Cl_I \cdot Q_{dif} \cdot Q}{\alpha_B \cdot Cl_I \cdot Q_{dif} + Cl_I \cdot Q + Q_{dif} \cdot Q} \quad \text{(Eq. I-14)}$$

Although these relationships (Eqs. I-8 to -14) describe the influence of serum protein binding on hepatic clearance, the influence of protein binding on drug elimination in other organs is expected to be similar. The perfusion-limited concept can be incorporated when describing the clearance of drugs in specific organs. For example, renal clearance (Cl_R) has been described by the following equation (227,277):

$$Cl_R = (\alpha \cdot GFR + \frac{\alpha_B \cdot Cl_{I,K} \cdot Q_K}{\alpha_B \cdot Cl_{I,K} + Q_K}) \cdot (1 - FR) \quad \text{(Eq. I-15)}$$

where GFR is the glomerular filtration rate, $Cl_{I,K}$ is the intrinsic ability to transport drug across the tubular membrane, relating the rate of secretion to the unbound drug concentration at the transport site, Q_K is the blood flow to the transport site, and FR is the fraction of drug filtered and secreted that is reabsorbed. Analogous to the hepatic clearance equations, the model predicts that for low kidney extraction drugs ($\alpha_B \cdot Cl_{I,K} >> Q_K$) secretion is proportional to the unbound fraction of drug in blood at the transport site:

$$Cl_T = (\alpha \cdot GFR + \alpha_B \cdot Cl_{I,K}) \cdot (1 - FR) \quad . \qquad \text{(Eq. I-16)}$$

When the ability to secrete the drug is high ($\alpha_B \cdot Cl_{I,K} >> Q_K$), the secretion is limited by the rate of delivery of drug to the secretory site, and the renal clearance then becomes:

$$Cl_R = Q_K \cdot (1 - FR) \quad . \qquad \text{(Eq. I-17)}$$

I-2. Protein Binding and Pharmacological Response

Pharmacological response is generally correlated with the degree or the rate of drug-receptor interaction (14,291), which is in turn dependent on the concentration of active species of a given drug at a receptor site. Because the drug concentration at a receptor site is in equilibrium with the concentration in plasma at steady state, plasma concentrations are usually used for clinical evaluation and monitoring purposes.

Because drug bound to macromolecules in plasma cannot readily diffuse into tissues, the unbound drug concentration in plasma is commonly believed to better represent the active concentration at a receptor site and, therefore, the pharmacological response. Although the notion is widely accepted, supporting evidence in the literature is limited.

Evidence has been presented by Anton (11) that the albumin-bound fraction of sulfonamides is devoid of antibacterial action *in vitro*. The unbound drug concentration of sulfonamides in the presence of albumin was always found to be the same as the concentration of an aqueous solution with the same antibacterial activity, regardless of the unbound fraction values of the drug. Similar findings have been reported for other antibiotics (299). Pharmacological activity of nortriptyline (42) and dipyridamole (308) *in vitro* have also been reported to be decreased by the addition of binding proteins.

McDevitt *et al* (246) reported that the unbound concentration of

propranolol in plasma was better correlated with the *in vivo* effect than with the total concentration in plasma. Yacobi *et al* (419), however, reported that there was no correlation of anticoagulant activity with either total and unbound concentration of warfarin or S-warfarin, but the variation in the concentration-effect relationship was smaller using unbound concentration than using total concentration. These observations support the clinical impression that the unbound drug level is better correlated with effect or toxicity (41).

One drug may alter the binding of another by competing for the same binding site, a drug interaction called *displacement*. Displacement has been used to demonstrate that unbound drug concentration is a better correlate of pharmacological effect. Trenk and Jähnchen (378) showed that the total plasma concentration of phenprocoumon needed to inhibit prothrombin complex synthesis rate by 50% decreased during coadministration of tolbutamide, a plasma protein displacer of phenprocoumon, while the concentration of unbound phenprocoumon needed to achieve the same effect did not change. Shoeman and Azarnoff (346), on the other hand, using phenylbutazone to displace phenytoin from plasma protein binding sites, changed the concentration-response curves for both unbound and total plasma concentration of phenytoin.

I-3. Binding Proteins

Drugs bind to various serum proteins; each of the serum proteins has its own characteristics. Drug binding proteins which have been identified are albumin, α_1-acid glycoprotein, lipoproteins, and some globulins.

I-3-a. Albumin

Albumin among the plasma proteins is undoubtedly the most important carrier for drugs and other small molecules. It binds drugs like digitoxin, warfarin, dicumarol, indomethacin, tolbutamide, phenylbutazone, diazepam, salicylate, sulfadimethoxine, phenytoin, and valproic acid among others, in addition to endogenous substances such as bilirubin, fatty acids, L-tryptophan, and cholic acid (113).

Albumin is a protein that contains 584 amino acids; its calculated molecular weight is 66248.3. It is highly water soluble and has 18 net negative charges at physiological pH. It is also characterized by the lack of a carbohydrate region common to many other plasma proteins (296).

The normal level of albumin in blood is 630 ± 53 μM. On a relative scale, albumin represents 60 ± 4% of the total amount of serum proteins. Only about two fifths of the albumin in the body is found within the vascular system at any one time. The remainder is located extravascularly. Two tissues containing large amounts of extravascular

albumin are skin and muscles, containing 18% and 15% of the body's share of albumin, respectively (296).

Although the importance of albumin as a drug binding protein is indisputable, it may well have been overstated. For example, in early studies, albumin was reported to be the major binding protein for drugs such as disopyramide (70) and quinidine (271), but more recently, it has been reported that both disopyramide and quinidine mainly bind to α_1-acid glycoprotein (117,231,273,305). The early reports can in part be explained by the fact that isolated crystalline albumin contains considerable inclusions of mother liquid, which in turn contains proteins such as transferrin and α_1-acid glycoprotein (157,231). In addition, the importance of α_1-acid glycoprotein as a drug binding protein was not realized until recently, while albumin has been recognized as the most plentiful and familiar binding protein in plasma for years.

I-3-b. α_1-Acid Glycoprotein

The importance of α_1-acid glycoprotein as a binding protein in plasma has been increasingly realized in recent years. There are a number of basic drugs and steroids, which are listed in Table I-1, that have been found to bind to α_1-acid glycoprotein.

α_1-Acid glycoprotein, a globulin with a molecular weight of 40,000, is characterized by its high water solubility at physiological pH, an isoelectric point at pH 2.7, and a very high carbohydrate content (45%) accounting for approximately 10% of all carbohydrate associated with

Table I-1. DRUGS REPORTED TO BIND TO α_1-ACID GLYCOPROTEIN

Drug	Reference	Drug	Reference
Alprenolol	303	Meperidine	38,264
Amitriptyline	52	Methadone	319
Aprindine	373	Moxaprindine	373
Bupivicaine	297,306	Nortriptyline	42,52
Cortexone	196	Perazine	52,336
Cortisol	196	Perphenazine	384
Chlorpromazine	289,304,384	Phencyclidine	131
Desipramine	126,382	Prazosin	92,328
Desmethylperazine	92	Progesterone	121,196
Diazepam	324	Propranolol	138,304,331,341
Dipyridamole	208,270,368	Quinidine	117,273
Disopyramide	229,230,231,305	Testosterone	196
Etidocaine	306	Thioridazine	209
Fluphenazine	209	Thiothixene	209
Imipramine	50,209,363	Trifluoperazine	384
Lidocaine	305,306,321,323	Verapamil	92
Loxapine	209		

normal human proteins (337). The carbohydrate moiety of the protein consists of approximately 11% sialic acid, 8% galactose, 6% mannose, 14% hexosamine, and 1% fructose. Linked to the carboxylic group of aspartic acid of the protein core, the polysaccharide chain consists of the sequence mannose, N-acetylglucosamine, galactose, and sialic acid. The abundance of peripheral sialic acid is also a distinguishing property of α_1-acid glycoprotein. The amino acid sequence of human plasma α_1-acid glycoprotein was shown to possess significant similarity with the immunoglobulins (337,338).

α_1-Acid glycoprotein has been shown to be synthesized in the liver (253). Its distribution in extravascular space has not been reported. The lymph-to-plasma concentration ratio of the protein was found to be approximately that of albumin and ranged between 0.4 - 0.6 in dogs (122). The half-life of ^{131}I-labeled α_1-acid glycoprotein was found to be 5.5 days in man (398,412). However, it was reported that desialized protein has a half-life of only 2 min in rats; parenchymal cells of the liver selectively remove this modified protein (261). The terminal galactose appears to play the major role in the hepatic recognition of this and certain other desialized plasma glycoproteins (261,337,364).

The biological role of α_1-acid glycoprotein has not been well established. A number of studies and speculations relate α_1-acid glycoprotein to blood clotting mechanism (8,78,202,355,379), triglyceride metabolism (361), phagocytosis (381), growth stimulation (236), immunosuppression (31), and spacing of collagen fibers from soluble collagen (114). The physiological consequence of changing α_1-acid glycoprotein

levels needs to be further studied.

Drug binding to α_1-acid glycoprotein has been shown to be inhibited in blood from Vacutainer tubes (43,79,153,302). The inhibitor appears to be tris-butoxyethyl phosphate ester (TBEP; 43). Selective displacement by the plasticizer has been reported for every α_1-acid glycoprotein-bound drug studied (301) and becomes an interesting characteristic for α_1-acid glycoprotein as a binding protein.

I-3-c. Other Binding Proteins

Drugs bind to a variety of plasma proteins in addition to albumin and α_1-acid glycoprotein. For example, globulins and lipoproteins can sometimes contribute significantly to the serum binding of drugs.

Lipoproteins binding a number of drugs such as chlorpromazine (33, 380,384), imipramine (33), quinidine (271,272), tetracycline (309), trifluoperazine (380,384), perphenazine (384), reserpine (67), clofibrate (380), and propranolol (138,380). Globulins are important for some drugs such as pancuronium (375), tubocurarine (25,75), and methadone (182,284).

Specific binders of various hormones also exist in plasma. For example, corticosteroids such as corticosterone and cortisol (67) bind to transcortin, which shows a strong affinity but low capacity for the bound drugs. There are also progesterone-binding globulins and testosterone-estradiol-binding globulins that bind the specific drugs more

tightly than transcortin (399). Except for artificial hormones, drug in general do not binding to these proteins.

I-4. Variation in Serum Protein Binding

Serum protein binding of drugs is altered by a number of pathological and physiological factors as well as by the interaction with some endogenous and exogenous substrates. The variations and the possible mechanisms causing the variations are reviewed in this section. Influences of altering binding capacity, affinity, and drug concentration on the extent of drug binding are also discussed.

I-4-a. Binding Affinity and Capacity

Unbound fraction (α) is a function of binding affinity, binding capacity, and unbound drug concentration (Cu). As defined, the unbound fraction is:

$$\alpha = \frac{Cu}{C_T} \qquad \text{(Eq. I-18)}$$

where C_T is the total drug concetnration, which is the sum of the concentrations of drug bound to various binding sites and unbound drug concentration. Therefore, Eq. I-18 can be rewritten as:

$$\alpha = \frac{Cu}{Cu + \Sigma Cb_i} \quad . \qquad \text{(Eq. I-19)}$$

If the binding at various sites on the same proteins or on different proteins follows the law of mass action, each Cb_i can be expressed by its binding dissociation constant (Kd_i), total number of binding sites (Pt_i), and unbound drug concentration:

$$Cb_i = \frac{Pt_i \cdot Cu}{Kd_i + Cu} \quad . \qquad \text{(Eq. I-20)}$$

Combining Eqs. I-19 and -20 gives:

$$\alpha = \frac{1}{1 + \Sigma \frac{Pt_i}{Kd_i + Cu}} \quad . \qquad \text{(Eq. I-21)}$$

This equation is useful to predict the unbound fraction when the binding capacity or binding affinity changes.

The number of binding sites varies under a number of circumstances. For example, serum albumin concentration has been reported as decreased in liver diseases, renal diseases, burns, surgery, gastrointestinal diseases, and other physiological or pathological conditions (184); an increase in α_1-acid glycoprotein in serum has been reported in diseases and/or conditions such as cancer, acute myocardial infarction, trauma, surgery, burns, or inflammation (220,337). An alteration in binding protein concentration results in a direct change in the number of binding sites. The binding of endogenous or exogenous substances to drug-binding proteins can indirectly cause an alteration in the number of binding sites. A more detailed discussion of factors that change the serum protein binding of drugs will be given in Sections I-4-c to -e.

The influence of binding capacity on the unbound fraction can be readily demonstrated for drugs with only one class of binding sites; the unbound fraction is:

$$\alpha = \frac{Cu + Kd}{Cu + Kd + Pt} \quad . \qquad \text{(Eq. I-22)}$$

As long as the concentration of binding sites is larger than the sum of the unbound drug concentration and the dissociation constant, the unbound fraction is inversely proportional to an alteration in concentration of binding sites. At high unbound fraction values (0.5-1.0) the effect of changes in the binding site concentration is less significant.

Alteration of the binding capacity of a single class of binding sites, however, does not necessarily change the unbound fraction significantly when multiple classes of binding sites are present. This is best demonstrated for a drug with two classes of binding sites in serum; one with low affinity and high capacity, the other with high affinity but low capacity. For example, a drug could bind to both albumin and α_1-acid glycoprotein. The serum concentrations of these binding proteins are 600 μM and 20 μM, respectively. Assuming one binding site per molecule and a dissociation constant of 1 μM for α_1-acid glycoprotein and 100 μM for albumin, the influence of changing binding capacity of the albumin and α_1-acid glycoprotein on the unbound fraction is shown in Fig. I-1.

As implied in Eq. I-21 and shown in Fig. I-1, unbound drug concentration determines the relative importance of the binding proteins. With the specified parameters, the binding to α_1-acid glycoprotein dominates at 0.1 μM unbound drug. Alteration in α_1-acid glycoprotein results in a large change in the unbound fraction; whereas changes in albumin concentration to the same relative extent elicits small changes in the unbound fraction, as shown in the lower panel of Fig. I-1. At a

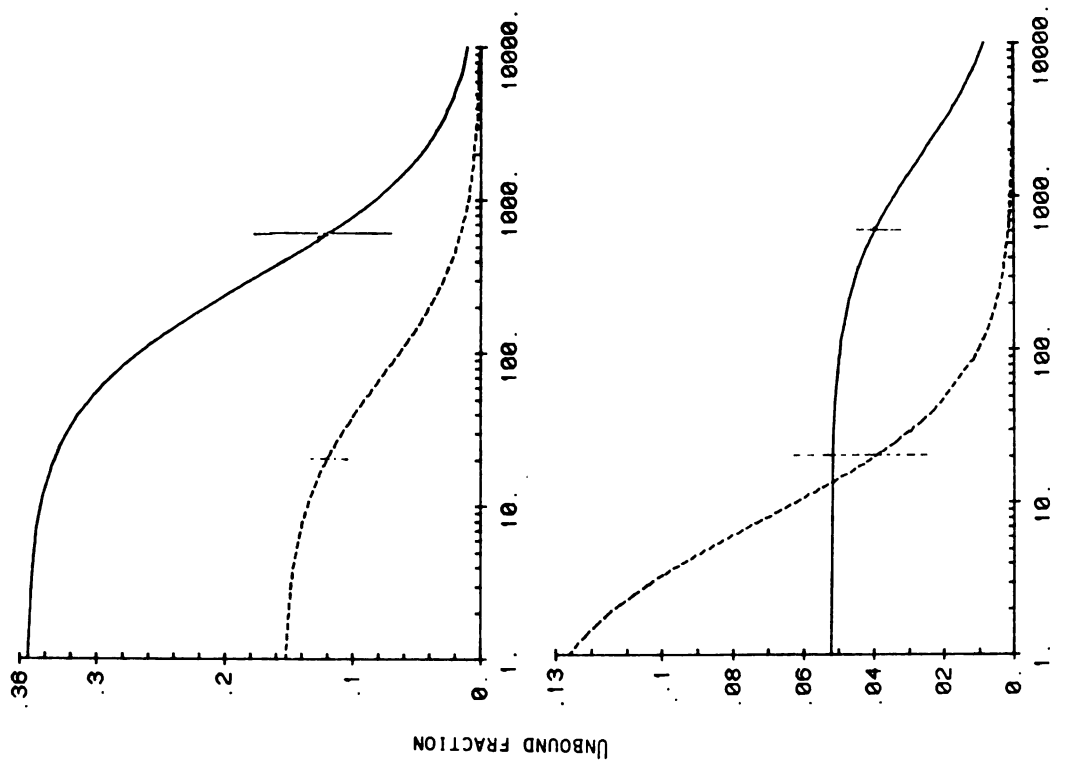


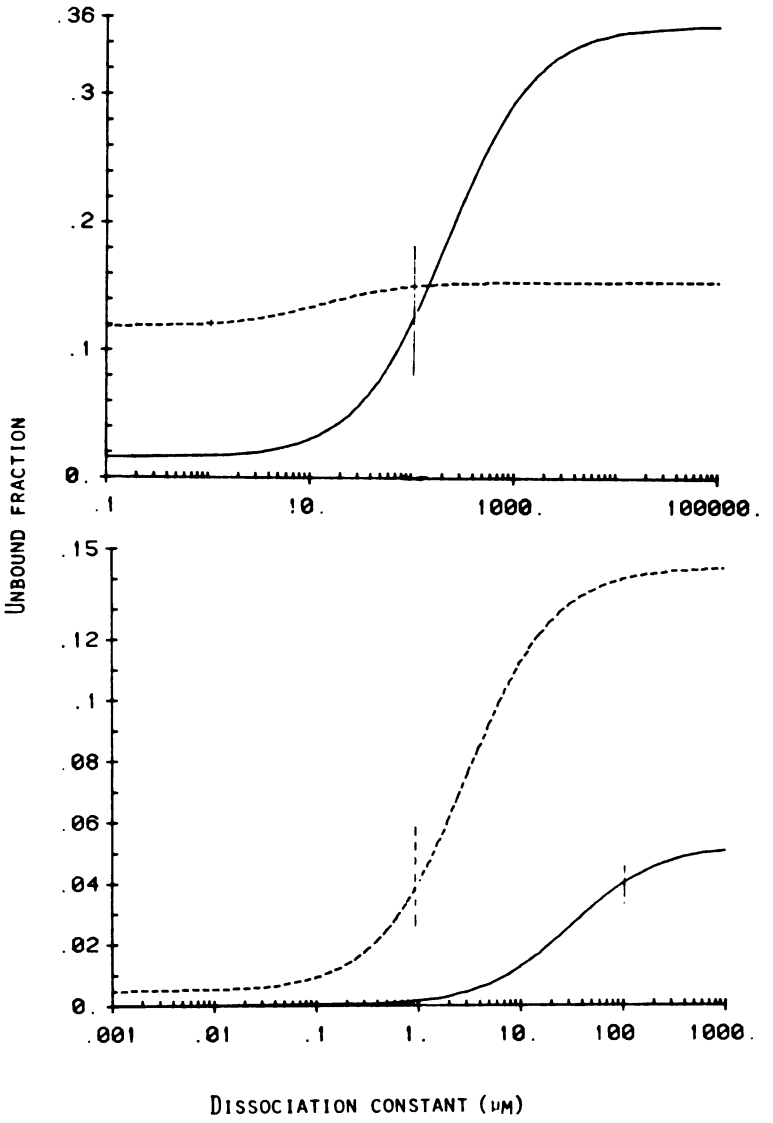
Fig. I-1. Influence of binding protein concentration on the unbound fraction of drug in serum. The dissociation constant for drug-albumin binding is 100 μM and for drug-α_1-acid glycoprotein is 1 μM. *Upper panel.* (——): Dependence of the unbound fraction on the concentration of albumin at an unbound drug concentration of 10 μM, keeping the α_1-acid glycoprotein concentration constant (20 μM). The vertical line at 600 μM indicates the alteration of the unbound fraction when albumin concentration is increased to 1200 μM and decreased to 300 μM. (---): Dependence of the unbound fraction of 10 μM unbound drug on the concentration of α_1-acid glycoprotein, keeping the albumin concentration constant (600 μM). The vertical line at 20 μM indicates the alteration of the unbound fraction when α_1-acid glycoprotein concentration is increased to 40 μM and decreased to 10 μM. *Lower panel.* (——): Dependence of the unbound fraction of 0.1 μM unbound drug on the concentration of albumin, keeping the α_1-acid glycoprotein concentration constant (20 μM). (---): Dependence of the unbound fraction of 0.1 μM unbound drug on the concentration of α_1-acid glycoprotein, keeping the albumin concentration constant (600 μM).

concentration of 10 μM unbound drug, on the other hand, the binding to albumin dominates, mainly because the α_1-acid glycoprotein is essentially saturated at a wide range of α_1-acid glycoprotein concentration. Albumin, although it does not bind the drug as strongly as α_1-acid glycoprotein in this example, becomes the major binding protein because of its abundance.

Binding affinity of serum proteins may be altered in pathological conditions such as uremia (205,216,345) or by competitive or noncompetitive inhibition by endogenous or exogenous compounds. An alteration of binding affinity will cause changes in the unbound fraction, provided the dissociation constant is larger than or of the same magnitude as the unbound drug concentration and smaller than the concentration of binding sites, as seen from Eq. I-22.

If multiple classes of binding sites are involved, the same principle can be applied to each individual site. An example is shown in Fig. I-2. Changes in the binding affinity of α_1-acid glycoprotein make small differences in the unbound fraction when the unbound drug concentration is much larger (10 μM) than its dissociation constant (1 μM; upper panel). When the unbound drug concentration is low (0.1 μM; lower panel) in comparison with the dissociation constant of either class of binding sites, the α_1-acid glycoprotein becomes the major binding protein for the drug. Changes in the binding affinity of α_1-acid glycoprotein make significant differences in the unbound fraction.

Fig. 1-2. Influence of binding affinity on the unbound fraction of drug that binds to albumin and α_1-acid glycoprotein. The concentration of albumin is assumed to be 600 μM and the drug-albumin dissociation constant to be 100 μM. Concentration of α_1-acid glycoprotein is assumed to be 20 μM and the drug-α_1-acid glycoprotein dissociation constant to be 1 μM. *Upper panel.* Dependence of the unbound fraction on the drug-albumin binding affinity at an unbound drug concentration of 10 μM, keeping the dissociation constant of drug-α_1-acid glycoprotein binding unchanged (——). The vertical line at 100 μM indicates alteration of the unbound fraction when the drug-albumin dissociation constant is increased to 200 μM and decreased to 50 μM. (---): Dependence of the unbound fraction on the drug-α_1-acid glycoprotein binding affinity at an unbound drug concentration of 10 μM, keeping the dissociation constant of drug-albumin binding unchanged. The vertical line at 1 μM indicates alteration of the unbound fraction when the drug-α_1-acid glycoprotein dissociation constant is increased to 2 μM and decreased to 0.5 μM. *Lower panel.* (——): Dependence of the unbound fraction on the drug-albumin binding affinity at an unbound drug concentration of 0.1 μM, keeping the dissociation constant of drug-α_1-acid glycoprotein binding unchanged. (---): Dependence of the unbound fraction on the drug-α_1-acid glycoprotein binding affinity at an unbound drug concentration of 0.1 μM, keeping the drug-albumin dissociation constant unchanged.



I-4-b. Drug Concentration

Drug concentration is one of the factors that determine the unbound fraction, as shown in Eq. I-21. The concentration-dependent unbound fraction has been shown for drugs like salicylate (120), disopyramide (89,162,190,229,251,370), and prednisolone (318,320) in the therapeutic range. Most other drugs, however, have a relatively constant unbound fraction at therapeutic concentrations.

The concentration dependency of the unbound fraction is simulated in Fig. I-3, assuming two classes of binding sites, one with high capacity and low affinity, the other with low capacity and high affinity. In this simulation, the unbound fraction shows concentration dependency at unbound concentrations above 1 μM, until all the binding sites are saturated when the unbound fraction approaches 1. It is the relative value of the therapeutic concentration range of the drug in comparison to the dissociation constants that determines whether concentration-dependent binding will be seen. Theoretically, all drugs with significant binding show concentration-dependent binding at high drug concentrations; a drug with linear binding at therapeutic concentrations may show decreased binding at elevated concentrations, i.e., during the distribution phase or in an overdose situation.

I-4-c. Disease States

Protein binding of various drugs are changed in a number of disease states. The binding alteration is generally associated with changes in

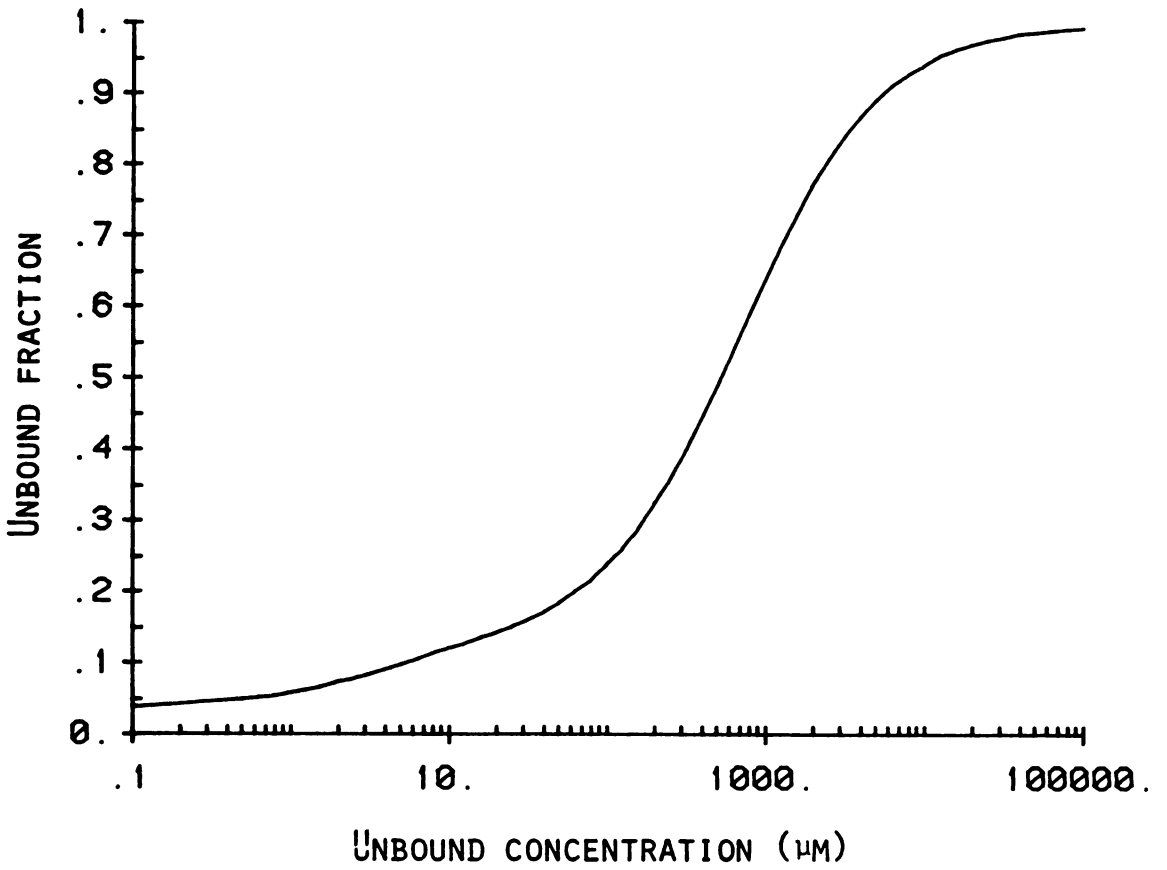


Fig. I-3. Relationship between unbound fraction of drug in serum and the unbound drug concentration. Binding proteins are assumed to be 600 μM albumin containing a single binding site, with a dissociation constant of 100 μM; and 20 μM α_1-acid glycoprotein containing a single binding site with a dissociation constant of 1 μM.

serum protein concentration and accumulation of endogenous compounds such as free fatty acids and bilirubin, together with unknown substances that may competitively displace drug from serum proteins. Binding alteration is most often reported in patients with liver or kidney diseases.

Liver diseases. The liver is the organ responsible for albumin synthesis as well as bilirubin degradation. Hepatic dysfunction, therefore, often results in hypoalbuminemia (351) and hyperbilirubinemia, the two major factors causing decreased drug binding of most of the drugs listed in Table I-2. Other unidentified factors have also been suggested to be responsible for the decreased binding (49,172,204,362,392,404), as hypoalbuminemia and hyperbilirubinemia can only explain part of the observed binding changes. For example, Kober *et al* (204) postulated an unknown binding inhibitor present in liver disease that inhibits the binding of diazepam and salicylate, but not warfarin, and which can be removed by charcoal treatment at pH 3.0.

Despite the fact that hypoalbuminemia and hyperbilirubinemia commonly occur in the course of liver disease, albumin-bound drugs do not necessary show altered binding in liver diseases. For example, warfarin is a drug known to bind to albumin and to be displaced by bilirubin (349), but its serum protein binding has been reported to be unchanged in liver disease (204,405) even when the bilirubin level is higher than in patients with normal hepatic function (405). Other studies have reported unchanged binding of phenytoin (3) and carbamazepine (167) in liver disease. The reasons for differences in the effect

Table I-2. EXAMPLES OF DRUGS WITH DECREASED SERUM PROTEIN BINDING IN LIVER DISEASE

Drug	Patient Condition	*Hypoalbuminemia	*Hyperbilirubinemia	Reference
Amylbarbital	Chronic hepatitis	✓		245
Azaproprazone			✓	172
d-Propranolol		✓		49
Salicylate		✓		204,295
Sulfadiazine		✓	✓	53
Diazepam	Cirrhotic			203,204
Etomidate		✓		62
Prazosin		✓		328
Propranolol				413
Theophylline		✓	✓	238
Thiopental		✓		127
Dapsone	Alcoholic	✓		3
Diazepam		✓		374
Fluorescein		✓		3
Phenylbutazone		✓	✓	53,392
Quinidine		✓		3
Salicylate		✓	✓	53
Tolbutamide		✓		374
Triamterene		✓		3
Phenytoin	Acute viral hepatitis	✓	✓	37
Tolbutamide			✓	404
Sulfadiazine	Active hepatitis		✓	392
Salicylate	Inactive hepatitis	✓		392
Salicylate	Cutaneous hepatic prophyria	✓		362
Phenytoin	(Mixed)	✓	✓	166,285
Morphine		✓	✓	285

*Reported causes of decreased binding.

of liver disease on serum protein binding of drugs are not well understood and need further investigation.

α_1-Acid glycoprotein concentration in serum may increase or decrease in liver diseases, with the average level found not to be statistically significantly different from that of normal subjects (373). Drugs binding to α_1-acid glycoprotein, therefore, usually show a more variable unbound fraction in patient serum, but with unaltered average values. For example, aprindine binding in serum was found to vary with serum α_1-acid glycoprotein levels in patients with liver disease who had normal average α_1-acid glycoprotein values; meperidine and lidocaine also showed insignificant binding changes in patients with liver disease compared to normal subjects (247,403). The major binding protein for both of these drugs is α_1-acid glycoprotein (Table I-1).

d-Tubocurarine binding is also unaltered in liver diseases (125). Because tubocurarine binds to globulin as well as albumin (75), an increase in the globulin levels compensating for a decrease in the albumin level (36) is thought to be an explanation.

Renal diseases. The influence of uremia on drug binding to serum proteins has been studied extensively, but the mechanism of binding alteration in renal disease is still not well established. As shown in Table I-3, a number of drugs show decreased binding in renal disease, while other drugs show unchanged binding in renal failure patients. The discrepancy appears to be due to binding to different

Table I-3. BINDING OF DRUGS TO SERUM PROTEINS FROM PATIENTS WITH POOR RENAL FUNCTION

Drug	*Binding	Reference	Drug	Binding	Reference
Aprindine	↔	373	Moxalactam	↓	298
Azapropazone	↓	172	Moxaprindine	↔	373
Bilirubin	↓	282	Nitrofurantoin	↓	80
Carbamazepine	↔	167	Papaverine	↓	29
Cephalothin	↓	80	Penicillin G	↓	80
Cephazolin	↓	80	Pentobarbital	↓	106
Chloramphenicol	↔	80	Phenylbutazone	↓	9,29,262,159
Chlorpromazine	↔	304	Phenytoin	↓	39,81,106,146, 149,166,235, 274,275,276, 285,317,345
Clofibrate	↓	149			
Cloxacillin	↓	80			
Dapsone	↔	80,316	Piretanide	↓	107
Desipramine	↔	317	Prazosin	↓	328
Diazepam	↓	142,205,350	Propranolol	↔	304
Diazoxide	↓	286	Quinidine	↓	2
Dicloxacillin	↓	80,81	Quinidine	↔	2,197,316
Diflunisal	↓	385	Quinidine	↑	29
Digitoxin	↓	29,81,205, 210,345,366	Salicylate	↓	9,81,108,295, 350
Digitoxin	↔	365	Sulfadiazine	↓	9,40
Disopyramide	↔	5	Sulfadimidine	↓	80
Etomidate	↓	62	Sulfametoxine	↓	350
Fluorescein	↓	2,316	Sulfamethazine	↓	80
Furosemide	↓	10	Sulfamethoxazole	↓	80,81
Indomethacin	↔	350	Thiopental	↓	9,127
Lidocaine	↔	142	Triamterene	↓	316
Maprotiline	↔	235	Valproic acid	↓	51,54,147
Methyldigoxin	↓	210	Verapamil	↔	193
Morphine	↓	285	Warfarin	↓	20,22,29,350
Metolazone	↓	377	Zomepirac	↓	311
Methyl orange	↓	59,102			

*↔ = Normal binding; ↓ = Decreased binding; ↑ = Increased binding.

proteins; albumin-drug binding is generally impaired in renal disease, while binding to α_1-acid glycoprotein is unaltered. The drugs in Table I-3 that show unaltered binding, such as quinidine, desipramine, lidocaine, disopyramide, aprindine, moxaprindine, chlorpromazine, propranolol, and verapamil, are known to bind to α_1-acid glycoprotein. These drugs usually demonstrate a more variable, but on the average, unaltered binding, depending upon the serum level of α_1-acid glycoprotein (142, 193,304,373).

Renal disease is commonly associated with a significant decrease in albumin and total serum protein concentrations (63). Hypoalbuminemia has been believed to be a partial reason for decreased binding in renal failure patients. Gugler *et al* (145,146) reported an excellent correlation between albumin concentration and drug binding in patients with nephrotic syndrome. Correlations between binding and albumin concentration in renal failure patients have also been reported in studies of diazoxide (286), morphine (285), etomidate (62), and diazepam (142). However, either no correlation or a poor one between the degree of binding and albumin concentration in renal failure patients has been reported in studies of valproic acid (147), phenytoin (285, 317), azapropazone (172), and thiopental (127).

Creatinine clearance, serum creatinine, blood urea nitrogen, and uric acid are used to quantitate renal function. These biochemical parameters were found to correlate with binding of drugs such as valproic acid (51,147), diazoxide (286), azapropazone (172), thiopental (127), warfarin (20), phenylbutazone (262), and in some studies, of

phenytoin (275,285,317), but not in other studies of phenytoin (102), and in studies of morphine (285). The correlation between renal function and drug binding is consistent with the hypothesis that endogenous inhibitors decrease drug binding in patients with decreased renal function.

Craig *et al* (80,81) reported that the defect in the binding of drugs in uremic patients is 1) greater than can be accounted for by hypoalbuminemia alone; 2) unchanged by prolonged *in vitro* dialysis; 3) transferred in the protein fraction but not in the ultrafiltrate fraction of uremic serum; 4) corrected by successful kidney transplant (350); and 5) corrected by treatment with activated charcoal at low pH (51,350). These investigators, therefore, postulated that the binding defect is due to the accumulation of inhibitors. Sjöholm *et al* (350) found that the binding constant of isolated albumin from uremic patients is unaltered by dilution, but the binding constant of albumin in uremic serum is altered by dilution, which is again consistent with the hypothesis of endogenous inhibitors decreasing drug binding.

Bilirubin and free fatty acids are known to reduce the binding of albumin-bound drug; the *in vitro* adjustment of the serum level of these two substances in normal serum, however, does not decrease drug binding as much as the binding decreases in uremic serum (81,82). Attempts have been made to isolate unknown inhibitors and various investigators have reported the inhibitor to be an unknown carboxylic acid (95), or an unknown peptide (199,200). McNamara *et al* (248) examined the accumulation of endogenous substances and concluded that hippuric acid

and indican are at least two of the inhibitors.

Albumin isolated from normal and uremic serum has been subjected to amino acid determination. The results suggested differences in the composition of the albumin from these two groups (40,345). It has also been postulated that cyanate hydrolyzed from accumulated urea may carbamylate albumin and decrease drug-albumin binding (21,22,108). However, later studies concluded that the extent of carbamylation in uremic serum albumin can only slightly contribute to the binding alteration (22). The real nature of decreased binding in uremia is still to be explored.

Other diseases causing hypoalbuminemia. It has been reported that hypoalbuminemia occurs in the majority of malnourished individuals (kwashiorkor), the degree depending on the duration and severity of undernutrition (212). Serum protein binding of albumin-bound drugs such as phenylbutazone (213,214), salicylate (110), and thiopental (19) was found to be decreased in serum from patients with kwashiorkor.

Hypoalbuminemia is also commonly present in postburn patients because the permeability of the skin capillaries is altered with an increased passage of protein from plasma to the interstitial fluid (122). A reduced serum albumin concentration and reduced drug binding have been demonstrated for phenytoin (38,46), diazepam (50), and salicylate (38) in postburn patients.

A number of other diseases also cause a severe decrease in serum

albumin concentration: cancer, freezing, bone fractures, myocardial infarction, surgery, acute febrile infections, and acute injury (63, 184,277,376). Drug binding to serum albumin is, therefore, expected to be lower in these diseases. In addition, hyperthyroidism decreases serum albumin level and the degree of protein binding of warfarin (112).

Diseases increasing α_1*-acid glycoprotein level.* α_1-Acid glycoprotein has been known as an acute phase reactant; serum levels usually increase two- to four-fold during stressful disease entities (220, 337) such as cancer (18,72,354,397), acute myocardial infarction (4,26, 50,65,179,305,322,325,356), typhoid fever infection (45), ulcerative colitis (90,239,334,396), Crohn's disease (304,334,396), rheumatic disorders (93,94,304,373), trauma or surgery (17,104,111,117,267,305), burns (38,423), coronary artery disease (371), and inflammation (7,173, 244,304).

Increased serum concentration of α_1-acid glycoprotein has been known to be due to an increased synthesis of protein (173,244,267), rather than from a release of deposits in the body. After an acute incidence, serum concentration of α_1-acid glycoprotein usually increases rapidly, peaking in 4-12 days, followed by a slow decrease (4,50, 104,179,356). During the elevation of α_1-acid glycoprotein concentration in serum, an increased serum drug binding is predicted, as shown in Section I-4-a.

Lidocaine binding has been found to be increased in patients with myocardial infarction (26,305,322,325), in epileptic patients with

elevated α_1-acid glycoprotein levels (323), in postburn patients (38), and in trauma patients (104). Propranolol binding was higher than in normal subjects in patients with Crohn's disease, inflammatory arthritis (304), surgery (111), and burns (38). Imipramine binding was increased in acute myocardial infarction (50), and postburn patients (38). Likewise, binding was increased for drugs like aprindine, moxaprindine in rheumatic patients (373), quinidine in patients after surgery (117), meperidine in postburn patients (38), and chlorpromazine in patients with Crohn's disease or inflammatory arthritis (304). In all cases, the serum drug binding was found to correlate with serum levels of α_1-acid glycoprotein.

I-4-d. Physiological Variation

Geriatric age group. Serum protein concentrations are known to change with advanced age, the pattern being a fall in albumin level with a rise in γ-globulin concentration (415). The levels of α_1-acid glycoprotein are reported to be higher in the elderly (94). Although the differences in protein concentration, approximately 10-20%, are statistically significant, they are not large enough to produce an important difference in drug binding.

Meperidine, a drug that mainly binds to α_1-acid glycoprotein (264), has been shown to exhibit no differences in serum protein binding between young and old subjects (64). It has also been shown that there is a positive correlation between unbound meperidine fraction in serum and age (242). These observations are contradictory to what would be

expected from a slight increase in serum α_1-acid glycoprotein levels in the elderly. For albumin-bound drug, insignificant differences in binding to serum from different age groups were found for diazepam (141, 203), phenytoin (30,155), benzylpenicillin, phenobarbital (30), salicylate, sulfadiazine, and phenylbutazone (393). Age-dependent binding has been reported for warfarin (154), diflunisal (385), and for phenytoin (293) and diazepam (1,394) in other studies.

Neonates and infants. Serum protein concentrations in neonates are different from normal adults, including drug binding proteins such as α_1-acid glycoprotein, and lipoproteins (137). Serum albumin concentrations in full-term newborns are slightly but significantly less (10-20%) than in adults (116,123,150,170,347,390), but the concentration slowly increases during the postnatal period. Premature neonates have a lower serum albumin level which is found to correlate with gestational age (137,170). α_1-Acid glycoprotein level in newborns is only approximately one-third of that for a normal adult (123,225,335,414), but increases rapidly in the first weeks after birth and reaches adult values by ten months (335). The β-lipoprotein level is only 5 to 42% (average 24%) of the mean adult level. After the first month of the neonatal period, the level increases and may even exceed the normal adult level by 9 months of age (137,165).

In addition to the lower binding protein concentration in serum, some other factors have been reported to affect drug-albumin binding in newborn infants. The presence of competing ligands such as bilirubin (71,105,116,216,312,313), free fatty acids and steroidal hormones

(116,211) has been suggested as a contributory factor to the observed reduction in neonates. A "fetal albumin" with decreased binding affinity to drugs has also been postulated (186,201,216,312,390,407). Neonatal albumin has been compared to the adult albumin by isoelectric focusing (391), and it was found that neonatal albumin contains only one of two components present in the adult albumin. The difference in the constituents of albumin might in part explain the difference in drug binding.

Examples of reduced binding of drugs to albumin in neonates are shown in Table I-4. For almost every albumin-bound drug studied, a significantly lower binding was shown in newborn infants compared to normal adults. However, an insignificant difference in binding between neonates and adults has also been reported for cephalothin (312) and digitoxin (32). For drugs that bind to α_1-acid glycoprotein, such as quinidine (307), imipramine (312), meperidine (264), propranolol, and lidocaine (414), lower binding in neonates than in adults has also been reported; however, an increased binding has been reported for chlorpromazine in newborns (48), which cannot readily be explained.

Binding changes during pregnancy. The serum albumin level is known to decrease gradually during the course of pregnancy (88,123,139,150, 359). A gradual decrease has also been reported for α_1-acid glycoprotein in some studies (123,139,225,359). However, other studies have reported no significant change in α_1-acid glycoprotein serum level (340,414). A decreased binding has been demonstrated in late pregnancy for drugs such as sulfisoxazole, dexamethasone (88,363), phenytoin

Table I-4. EXAMPLES OF DRUGS SHOWING REDUCED BINDING IN NEONATES

Drug	Reference
α-Azidobenzylpenicillin	105
Benzylpenicillin	105
Bilirubin	186,211,180
Cephazolin	201
Chloramphenicol	215
Chlordiazepoxide	215
Diazepam	414
Diazoxide	312
Digitoxin	215
Furosemide	13
Meticillin	215,216
Nitrofurantoin	215
Paraaminosalicylic acid	215
Pentobarbital	347
Phenacetin	215
Phenobarbital	105,215
Phenytoin	44,105,116,211,215,312,313
Promethazine	215,216
Salicylic acid	48,211,215,347,390,407
Sulfadiazine	390
Sulfamethazine	48
Sulfamethoxazole	48
Sulfaphenazole	71
Thiopental	215,216

(66,88,330,363), salicylate (88,233,363), diazepam (88,363,414), phenobarbital (66), *d*-tubocurarine, metacurine, propranolol, lidocaine (414), but not bilirubin (363). Although the binding was shown to correlate well with binding protein concentration (66,88,330,414), the decrease of binding protein concentration was only considered to be part of the reason for decreased binding. Endogenous displacers such as free fatty acids and hormones (233,263,363) have been postulated to additionally reduce serum protein binding. For example, binding of drugs to serum from pregnant women increased upon treatment of serum with activated charcoal (363), which supports the notion that accumulation of endogenous inhibitors are one of the mechanisms for reduced binding in pregnant women. The serum level of transcortin, on the other hand, increases during pregnancy (333), which increases the binding of cortisol (332).

Binding changes during therapy with oral contraceptives. Changes in the serum protein pattern produced by oral contraceptives are comparable with those caused by pregnancy, although the latter entity has a more pronounced effect (139). A significant decrease in α_1-acid glycoprotein levels in women receiving oral contraceptives has been reported in some studies (139,359), while others find no change (414). Changes in the albumin level, on the other hand, are insignificant during oral contraceptive therapy (139,359). Oral contraceptives, which have the ability to decrease drug binding to a lesser degree than pregnancy, are shown to decrease the binding of salicylate (88), lidocaine (414), and diazepam (414) significantly, but insignificantly in studies of phenytoin (166, 330), *d*-tubocurarine, metocurine, propranolol (414), lidocaine (324), and nitrazepam (198). Serum levels of transcortin are found to be

higher after the use of estrogen (254,333,352). The binding of hydrocortisone (254) is also reported to be significantly increased.

Gender. A statistically significant difference in serum albumin concentration exists between men and women, but not in α_1-acid glycoprotein concentration (94,123,324). Despite the differences in albumin concentration, the difference in albumin-drug binding was not shown in studies of nitrazepam (178), phenytoin (166), *d*-tubocurarine, metocurine (414), diazepam (394, 414), and warfarin (418). Diazepam binding in serum was shown to be significantly higher in men than in women in some studies (1, 141, 324), but the difference could not solely be attributed to differences in albumin concentration. It might be due to the difference in serum levels of steroidal sex hormones or other endogenous drug binding inhibitors. Consistent with little-to-no differences in α_1-acid glycoprotein concentration between genders, no significant difference in protein binding of propranolol (414) or lidocaine (324,414) was found between men and women.

Free fatty acid level. Fasting (15,47,100,223), exercise (148,294), stress (223,294), adrenergic stimulation (15,100,116,148,223), and ACTH secretion (15,223,342) are reported to increase the concentration of serum free fatty acids, which are known to alter binding of many drugs (326,329,339,340,401). Warfarin binding is decreased by fasting (223), stress (223), exercise (148), and adrenaline administration (148) in experimental animals, as is phenytoin binding (116,148). Binding of valproic acid is reported to be lower in fasting volunteers (47).

Genetic variation. The magnitude of intrasubject variation has been shown to be relatively small compared to intersubject variation in the binding of warfarin to serum proteins (420). The binding affinity of warfarin to albumin has been found to be less variable within monozygotic twins than within dizygotic twins (402). Similarly, the variance of the binding fraction within dizygotic twins was significantly greater than monozygotic twins in a study of nortriptyline (6), although no difference was shown in a study of diazepam between monozygotic and dizygotic twins (1). The contributory factors of genetic variation are not known. Differences in warfarin binding to polymorphic variants of albumin (395) was one of the initial experiments revealing genetic variation in drug binding.

I-4-e. Drug Interactions

The extent of drug binding to serum proteins in influenced by the presence of other drugs which also binding to the same protein. Numerous studies in drug binding displacement have been published in the last two decades. The best known drugs which displace other serum albumin-bound drugs are those needed in high therapeutic concentrations and which are highly bound to albumin, such as salicylate, phenylbutazone, tolbutamide, and valproic acid. Valproic acid displaces phenytoin (83,85,119,124,260, 300) and diazepam (98,99) from serum albumin; salicylate decreases serum protein binding of phenytoin (73,115,276,234), indomethacin (422), furosemide (310), diflunisal (383), tolmetin (344), valproic acid (389), methotrexate (372), sulfadimethoxine, sulfamethoxypyridazine (12), and bilirubin (101,281); phenylbutazone decreases serum protein binding of

warfarin (287,351,416), phenytoin (234,268,346), naproxen (185), sulfaethylthiadiazole (11), furosemide (310), bilirubin (101), and cefazolin (61); tolbutamide decreases serum protein binding of furosemide (310), phenprocoumon (378), phenylbutazone (358), sulfaethylthiadiazole, sulfadimethoxine, and sulfamethoxypyridazine (12). Drugs whose molar therapeutic concentration is relatively low compared to the molarity of serum albumin do not displace other drugs effectively; for example, diazepam was reported not to interfere with the binding of carbamazepine (167).

A similar displacement interaction also occurs for drugs bound to α_1-acid glycoprotein. For example, quinidine, meperidine, and desipramine decrease the binding of bupivacaine (126); bupivacaine, disopyramide, and quinidine decrease the binding of lidocaine (250). Propranolol, however, does not displace lidocaine (250) because of its low therapeutic concentration.

At least five different binding sites have been located on human albumin (113). The binding of a ligand to a specific site may competitively inhibit drug binding to the same binding site or inhibit drug binding to the other sites noncompetitively. However, drugs binding to different sites on the same protein do not necessarily displace each other. For example, salicylate does not displace naproxen (185) or sulfaethylthiadiazole (12), whereas phenylbutazone does; tolbutamide does not interfere with binding of diflunisal (383) whereas salicylate or bilirubin does. All these drugs are known to bind to albumin.

Although protein binding in the presence of other drugs is

commonly observed to be decreased, enhanced serum protein binding by the presence of other ligands has also been reported. Tolmetin binding to serum albumin is increased in the presence of phenylbutazone, oleic acid, and acetaminophen (344). The unbound fractions of benzylpenicillin, cephalothin, and cefoxitin are also found to be lower at a high molar ratio of free fatty acid to albumin than with low ratios (369).

Altered serum protein binding has also been reported to be due to indirect drug interaction. Oral contraceptives change various serum protein concentrations and concentrations of endogenous binding inhibitors resulting in decreased drug binding for a number of drugs (Section I-4-d). The rate of synthesis of albumin and α_1-acid glycoprotein was shown to be decreased in the presence of phenylbutazone and salicylate in liver slices prepared from rats (174); this change in the rate of protein synthesis might decrease serum albumin and α_1-acid glycoprotein levels and drug binding. Furthermore, a single dose of sodium oleate induced a prolonged phenytoin binding reduction by an unknown mechanism in experimental rats (76). The drug interaction *in vivo* might affect serum drug binding in a more complicated way than that shown *in vitro*.

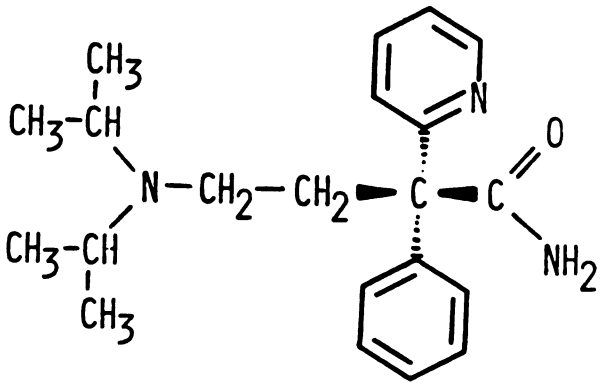
I-5. Disopyramide

Disopyramide is a synthetic drug developed in the early 1960's. Mokler and Van Arman (259) described its antiarrhythmic activity in 1962, Katz *et al* (192) reported the first clinical trial in 1963, and the drug was first marketed in France in 1969. In the United States, its oral usage was approved in 1977. The intravenous dosage form is, at present, only available as an investigational drug.

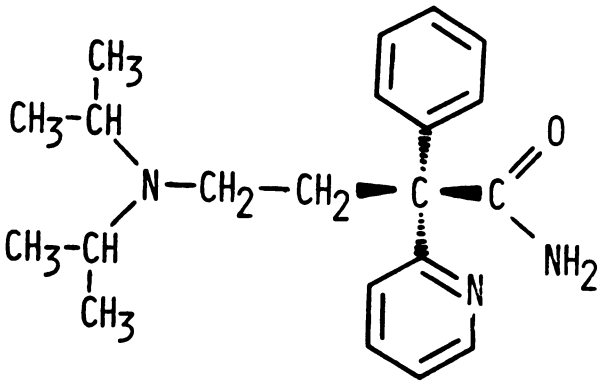
The physico-chemical, pharmacodynamic, and pharmacokinetic properties of disopyramide are reviewed in this section.

I-5-a. Physico-chemical Properties

Disopyramide (SC-7031, H-3292, Dicorantil, Ritmodan, Rythmodan, or 4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butyramide) is a colorless solid with a melting point of 94.5-95.0° (crystal from hexane). Disopyramide has a chiral center at the C-2 position, with resulting R- and S-enantiomers, as shown in Fig. I-4. R- and S-disopyramide have optical rotations of -19.4° and +18.9°, respectively (57); therefore, they are also named *l*-disopyramide and *d*-disopyramide, respectively. Disopyramide is soluble in chloroform, dichloromethane, acetone, acetonitrile, ethylacetate, ethylether, hot hexane, or acidic aqueous solutions. Its methanolic solution absorbs UV at 260 nm maximum. It has pKa of 8.36 in aqueous solution (69).



R-DISOPYRAMIDE



S-DISOPYRAMIDE

Fig. I-4. Chemical structures of R- and S-disopyramide.

I-5-b. Clinical Use

A large number of clinical investigations of disopyramide have been published in the last two decades which have been summarized in a few reviews (156,192,206). In general, disopyramide is useful to suppress ventricular and supraventriculat tachycardia, to reduce the frequency of atrial and ventricular premature beats, and to correct and prevent atrial fibrillation and flutter (91,151,168,169,241,353, 387,388). Disopyramide is, however, only approved for the treatment of ventricular arrhythmia in adults in the United States.

The anticholinergic action of disopyramide produces a significant incidence of dry mouth, constipation, blurred vision, urinary hesitancy, and, occasionally, urinary retention. Recently, serious and sometimes rapidly developing cardiac decompensation has been reported in patients receiving disopyramide, particularly in patients having preexisting cardiac function abnormalities (96,198,232,308,348,367). The greater negative inotropic effect of disopyramide than any other Class I antiarrhythmic agent (for example, procainamide and quinidine) has substantially limited its use clinically.

In a few studies (269,314), antiarrhythmic activity has been related to the disopyramide serum concentration. Concentrations between 3 to 6 mg/ℓ are estimated to be the desirable therapeutic range (156).

I-5-c. Pharmacodynamic Properties

Cardiac electrophysiologic effect. Sekiya and Vaughan Williams (343) first observed a concentration-dependent "quinidine-like" effect of disopyramide on rabbit atria action potential. Similar results were subsequently observed in canine Purkinje fibers or guinea pig papillary fibers. In short, disopyramide acts on sodium and calcium channels in the cell membrane (152), which decreases the maximum rate of phase 0 depolarization (86,152,207,217,219,343,421) and the rate of phase 4 depolarization (86,152,217). The amplitude of the action potential therefore decreases (86,152,217,343), with an increase in the duration of the action potential (86,152,207,217,19,421) and the refractory period (86,217, 219), and a decrease of the conduction velocity (86,217, 343,421). The effects have been reported to be potassium concentration dependent (86,207,219). Automaticity is also decreased by disopyramide (68,86,140,255,343,421). The mechanism is unknown, although a decreased adenosine 3',5'-cyclic phosphate content may be responsible (255). The electrophysiological effects of R- and S-disopyramide while similar have some important differences. The effect on the maximum rate of phase 0 depolarization and the conduction time is not different between R- and S-disopyramide (258). The isomers are also equally effective in prolonging the effective refractory period (57). However, S-disopyramide as well as racemic disopyramide increases the action potential duration whereas R-disopyramide decreases it or has no effect (258).

Anticholinergic effect. Anticholinergic activity of disopyramide, which is often observed clinically, has been demonstrated *in vitro*

(23,58,128,256). Disopyramide blocks the muscarinic receptors stereoselectively, S-disopyramide being about 3- to 4-fold more active than R-disopyramide in antagonizing the action of acetylcholine or physostigmine (128,256); but the activity of both enantiomers is only approximately 1/20 of that of atropine (23,256). At high drug concentrations (>20 μM), disopyramide will produce ganglionic blockade (58). The known metabolite of disopyramide in man, N-monodealkylated disopyramide, also elicits stereoselective anticholinergic effect; the S-enantiomer of the metabolite is about 3-fold more active than the R-enantiomer in blocking the action of acetylcholine (23,266).

Electrocardiographic effect. The electrocardiographic effect of disopyramide *in vivo* is complicated by its anticholinergic activity because the cholinergic system regulates the electrophysiological properties of both atria and ventricles (257). Disopyramide has been reported to decrease (35,421) or to have no effect (27) on the sinus rate. Its effect on sino-atrial conduction is reportedly not consistent among patients (221), neither is the effect on atrioventricular (A-V) nodal conduction. In some studies, an unaltered A-V conduction (60, 97,240) and an unaltered PR interval (168) are found; while in other studies, an increased A-V conduction time (35,421), consistent with an increased PR interval (27,89,218,315), has been reported. A slight but significant widening of the QRS duration which is associated with decreased conduction velocity in the ventricles (60,221,240,360,421) has commonly been found (27,89,96,168,169,218,221,232). A prolongation in QT_c interval has also been reported (16,27,89,96,164,168,169,218, 232,400) which reflects an increased refractory period (97,181,240,360).

Hemodynamic effect. Disopyramide produces a dose-dependent negative inotropic effect in experimental animals (68,89,140,243,259,265,421). Studies in man have confirmed this negative inotropic action, particularly in patients with heart disease or arrythmias following rapid intravenous injection (161,169,176,370,386,406). Cardiac output decreased in the first 20 minutes after an intravenous bolus administration of 2 mg/kg disopyramide (27,406). Some authors have reported a slight increase in blood pressure and systemic peripheral resistence (27,240) in patients with cardiac disease or arrhythmias after having administered 1 to 2 mg/kg disopyramide intravenously while others have observed no significant change in blood pressure (23,240). Studies in experimental animals have not been able to demonstrate significant changes in mean blood pressure after disopyramide administration (89,218,421). An increase in heart rate has been reported in patients and healthy subjects (164,176,370) after disopyramide administration; while in other studies with patients and animals, disopyramide was not found to alter the heart rate (89,176,240).

Activity against experimentally-induced arrhythmias. Intravenous doses ranging from 1 to 10 mg/kg, and oral doses ranging from 2 to 5 mg/kg of disopyramide, have been found to protect against aconitine induced fibrillation in dogs and rats (177,183). Intravenous doses of 1 to 5 mg/kg were additionally found to be effective in abolishing different types of ventricular arrhythmias in dogs with experimental myocardial infarction (218). In comparison with other antiarrhythmic drugs, disopyramide was more potent on a weight basis against aconitine or injury-induced atrial arrhythmias than quinidine, procainamide, or

lidocaine (23,89,259). Comparing the ability to abolish ventricular arrhythmias produced in dogs by coronary artery occlusion or ouabain intoxication, disopyramide was found to be more active than ajmaline, procainamide, lidocaine, phenytoin, or quinidine in several studies (77,89,259), but equally active with quinidine (259) or less active than lidocaine (23) in other experiments.

I-5-d. Pharmacokinetic Properties

Serum protein binding. The literature data describing disopyramide binding to human serum protein are divergent. Not only does the reported unbound fraction vary from study to study, but there is controversy with regard to which serum protein the drug primarily binds. However, the literature in general agrees that disopyramide serum protein binding is concentration-dependent (84,87,162,190,229,251).

Chien *et al* (70) first reported on the binding of disopyramide. They studied the binding of disopyramide by ultracentrifugation at concentrations of 5.4 to 21.8 mg/ℓ in 50% diluted human plasma or 350 μM human serum albumin in phosphate buffer. An unbound fraction of 69.3% to 77.7% was reported for diluted human plasma and 67.0% to 77.3% for the albumin solution; albumin was therefore concluded to be the major binding protein in human plasma. Piafsky *et al* (305) on the other hand reported an unbound fraction of 18.2% for cardiac post-surgery patients and 23.9% for healthy volunteers at 2 mg/ℓ. Because α_1-acid glycoprotein concentrations are higher in cardiac patients, it was suggested that α_1-acid glycoprotein is the major

binder of disopyramide in human serum. The importance of α_1-acid glycoprotein in disopyramide binding was further confirmed in the studies by Lima *et al* (229,231). The lower binding reported by Chien *et al* (70) could be explained by the high disopyramide concentration and diluted human plasma they used. In addition, a drug that binds to albumin with low affinity and to α_1-acid glycoprotein with high affinity may show albumin as the main binding protein at high drug concentration and α_1-acid glycoprotein as the main binding protein at low drug concentration. Contamination of most human albumin preparation with α_1-acid glycoprotein is an alternative explanation to the data found by Chien *et al* (70).

Hinderling *et al* (162) reported unbound fractions of disopyramide in blood bank plasma ranging from 50% at 1 mg/ℓ to 70% at 9.3 mg/ℓ. These results approximate those reported by Cunningham *et al* (84), which ranged from 32.4% at 0.38 mg/ℓ to 65.7% at 3.8 mg/ℓ, and those from Karim *et al* (190), which ranged from 48% at 1 mg/ℓ to 75% at 10 mg/ℓ. However, David *et al* (87) reported a much lower unbound fraction in healthy volunteers, ranging from 18% at 0.68 mg/ℓ to 64% at 7.5 mg/ℓ, which is consistent with results from Lima *et al* (153,229), who reported an unbound fraction ranging from 32% at 2 mg/ℓ to 58% at 6 mg/ℓ. The discrepancy cannot be readily explained. Hinderling *et al* (229) used pooled blood bank plasma which usually contains 20% of anticoagulant solution which might explain their observed low binding. Lima *et al* (229) found the binding to blood bank plasma to be lower than in normal serum. The unbound fraction of the donor plasma ranged from 34% at 0.34 mg/ℓ to 80% at 6.8 mg/ℓ, which is essentially

the same as that reported by Hinderling *et al* (162). Karim *et al* (190), on the other hand, did not specify the source of human plasma or blood sampling techniques in their studies. Cunningham *et al* (84) used pooled fresh normal human plasma in unspecified tubes containing heparin. The use of Vacutainers containing plasticizer TBEP which decreases disopyramide binding (153) might possibly explain the low disopyramide binding observed by Cunningham *et al* (84) or Karim *et al* (190). The importance of TBEP as an inhibitor of drug binding to α_1-acid glycoprotein was, however, not realized until after the reports by Karim *et al* (190) and Cunningham *et al* (84) were published. Data reported in recent years (5,87,229,251,253) may, therefore, better represent the *in vivo* binding of disopyramide as some of the problems involved with previous determinations have been avoided.

Meffin *et al* (251) reported a higher disopyramide binding in cardiac patients than in normal subjects. The unbound fraction in those patients ranged from 19.2% at 2 mg/ℓ to 45.6% at 8 mg/ℓ, similar to the values observed by Piafsky *et al* (305) in patients with myocardial infarction. The increased α_1-acid glycoprotein levels observed explain the increased disopyramide binding. Lima *et al* (229) also studied disopyramide binding in the therapeutic range to serum of patients with arrhythmias, and found binding to be higher than to serum from normal subjects.

Changes in pH minimally affect disopyramide binding. Binding is lower at pH 6.7 than at pH 7.4; on the other hand, no difference in the binding was observed between pH 7.4 and pH 8.0 (162). Temperature

has little effect on disopyramide binding as the binding is similar at 25°C and 37°C using equilibrium dialysis (162). Heparin and EDTA appear to have no effect on serum protein binding of disopyramide (153).

Disopyramide binding to serum proteins in a number of species has been compared. At low disopyramide concentrations (1-10 μM), the unbound fraction ranks as follows: rabbit (the highest), rat, cow, dog, monkey, guinea pig, sheep, man, horse (230). The interspecies difference of disopyramide binding could be due to genetic variation in the primary structure of α_1-acid glycoprotein (230), which is known to differ in molecular weight and carbohydrate content (337).

Distribution. Studies in rats and dogs show that disopyramide distributes widely in the body. High concentrations of disopyramide are found in liver, kidney, spleen, and lungs, whereas lipophilic tissues such as fat or brain have low concentrations of disopyramide (189,190). This is consistent with the low partition coefficient of disopyramide in *n*-octanol/water (70). Disopyramide concentration in myocardium (μg/g wet tissue) is approximately two- to four-fold higher than in plasma (μg/ml) over a wide range of doses in dogs (190, 292) and rats (189). Nonlinear uptake of disopyramide has been shown in lungs, eyes, adrenals, liver, and salivary glands in rats (189). The ratio of red blood cell drug concentration to unbound serum concentration is found to be concentration-independent and averages 1.13 in man (162). Studies in rats using radioactive disopyramide show a high degree of placental and breast milk transfer of disopyramide (188). Total radioactivity in plasma and in the brain are much higher

in the fetus in comparison to the mother. Concentration in the milk of lactating rats is also higher than the concentration found in plasma.

Metabolism. Metabolism of disopyramide has been studied in man, dog, and rat (187,189-191). The recovery of known metabolites (structures shown in Fig. I-5), together with unchanged disopyramide in urine and feces, account for the majority of the administered disopyramide in all species (85-95%). N-monodealkylated disopyramide, compound I in Fig. I-5, is the only known human metabolite, which accounts for 20% of the total administered dose in urine and 7% in feces (163,187,191). In rats the major metabolic pathway is aryl hydroxylation instead of N-dealkylation. Compounds V and VI in Fig. I-5 have been identified as the major metabolites, and are found in both urine and feces. In feces, however, they exist mainly as sulfate conjugates. Compounds I, III, and IV have also been detected, but only contribute to a small degree to the overall metabolism (189,191). N-dealkylation is the major metabolic pathway of disopyramide in the dog. The major metabolite is compound II or III. Compound II is unstable and becomes compound III *in vitro*. Compounds I, V, and VI were found as minor metabolies; compound IV was not detected in the dog (190,191).

Pharmacokinetic parameters. The pharmacokinetics of disopyramide have been widely studied in the last decade, and pharmacokinetic parameters in normal subjects and in various types of patients have been published. Although concentration-dependent serum protein

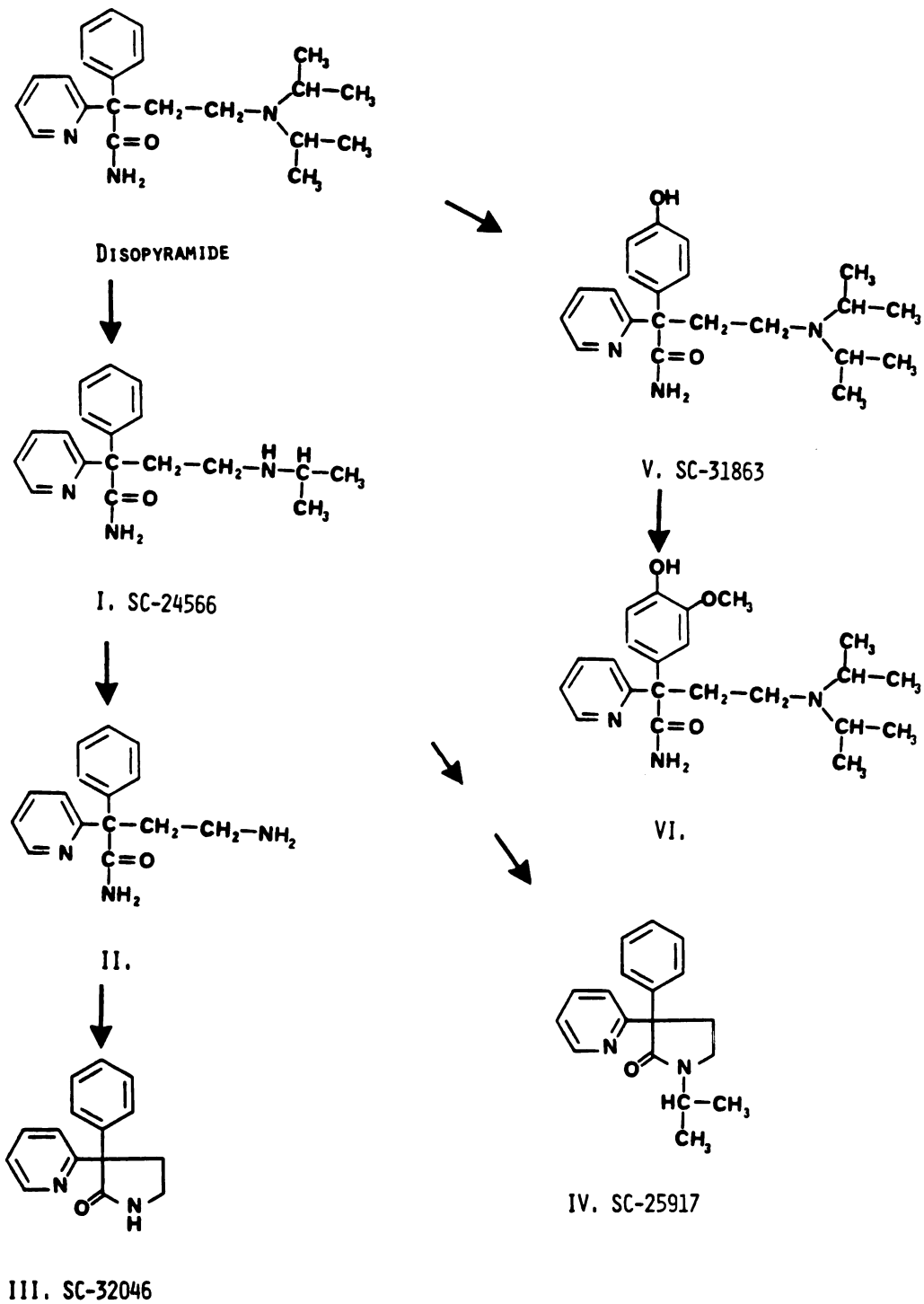


Figure I-5. Metabolism of disopyramide.

binding of disopyramide has been known for years, this has generally been disregarded when making parameter estimates of disopyramide disposition. Total disopyramide concentration in serum has generally been used to calculate pharmacokinetic parameters (55,56,103,162,171, 180,224,226). Because of concentration-dependent serum protein binding, the calculated pharmacokinetic parameters such as total body clearance, renal clearance, volume of distribution, half-life, and bioavailability, will depend upon the disopyramide dose given, the mode and rate of drug administration, sampling schedules, method of calculation, and the intra- and interindividual variability in protein binding. Disopyramide pharmacokinetic parameters with respect to total serum concentation are, therefore, time- and concentration-dependent variables. The results are difficult to interpret with values not easily compared between studies.

Unbound drug concentration-time data has been used to calculate pharmacokinetic parameters in a few studies (84,251,163,229), which are theoretically essentially independent of binding. However, some studies have estimated unbound drug concentrations from questionable unbound fractions (84,163), as discussed in the previous section. The calculated pharmacokinetic parameters in these studies are, therefore, of questionable value.

Unbound drug clearance of disopyramide was shown to be independent of dose and concentration in cardiac patients given multiple infusions, whereas total drug clearance was concentration-dependent (251). It was also shown that the area under the unbound disopyramide concentration-

time curve was directly proportional to the dose when four different oral doses were given to healthy volunteers, but not if the total serum drug concentration was used (229). These findings can well be explained by concentration-dependent serum protein binding based on the current concepts of drug elimination. Because disopyramide has a low extraction ratio in the eliminating organs, its blood clearance is proportional to the unbound fraction and the unbound clearance is constant (section I-1-b).

The unbound clearance of disopyramide in cardiac patients was 0.67 ℓ/min (251). Forty to sixty percent of disopyramide administered was excreted unchanged in the urine (84,163,168,187).

Based upon theoretical considerations, the unbound volume of distribution of a drug with concentration-dependent binding may also be concentration-dependent (section I-1-a). Meffin *et al* (251) reported a time-averaged unbound volume of distribution of 207.6 ℓ in patients given 2 mg/kg disopyramide over 15 min. The value is within the range in which a concentration-dependent unbound volume of distribution may be evident.

The half-life of unbound disopyramide concentration in serum has been reported to be 3.8 hr in cardiac patients (251) and 4.4 hr in normal subjects (229). The half-life is, however, likely concentration-dependent, as the unbound volume of distribution varies with the disopyramide concentration and the unbound clearance of disopyramide is concentration-independent.

I-5-e. Rationale as a Model Drug

To demonstrate that unbound drug concentration, and not total drug concentration, in serum best represents the pharmacological response, disopyramide was selected as a model drug because of the following characteristics: 1) the pharmacological response of disopyramide can be measured by electrocardiography *in vivo*; 2) disopyramide does not bind to rabbit serum protein and binding can be readily increased by injection of human α_1-acid glycoprotein, a high affinity binder of disopyramide; 3) disopyramide acts on the conduction system in the heart which is a well perfused tissue. Animal studies (189,190) suggest that serum drug concentration correlates with the drug concentration in the heart, and the time necessary to reach apparent equilibrium is only a few minutes; and, 4) there is no evidence to show that there are any active metabolites which may significantly contribute to the pharmacological response measurement *in vivo*.

In addition to what is listed above, R-disopyramide is a relatively low extraction ratio drug, while S-disopyramide is a relatively high extraction ratio drug at low concentrations in the rabbit (see Chapter III). This allows for determining differences in the effect of protein binding changes on drugs that have the same physicochemical properties but show different elimination characteristics.

Chapter II.

Materials and Procedures

II-1. Materials and Equipment

Drugs and injection solutions. Disopyramide phosphate capsules (Norpace^{R}), disopyramide primary standard, chlorodisopyramide, and N-monodealkylated disopyramide were obtained from G.D. Searle & Co. (Chicago, IL). Indocyanine green and its aqueous solvent for injection were obtained from Hynson, Westcott & Dunning, Inc. (Baltimore, MD). Normal saline solution for injection was obtained from Travenol Laboratories, Inc. (Deerfield, IL) or from Elkins-Sinn, Inc. (Cherry Hill, NJ). Heparin sodium (Liquaemin^{R} sodium) injection solution (1000 U/ml) was obtained from Organon, Inc. (West Orange, NJ). Sterile water for injection was obtained from Abbott Laboratories (North Chicago, IL).

Chemical reagents and solvents. Phosphoric acid (85%), reagent grade, was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Potassium phosphate, dibasic sodium phosphate heptahydrate, dichloromethane, methanol, and acetone were analytical grade and obtained from Mallinckrodt, Inc. (Paris, KY). Sodium hydroxide solution (50% w/w) and *d*-tartaric acid were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). *l*-Tartaric acid was obtained from Sigma Chemical Co. (St. Louis, MO). Tetrabutylammonium hydrogen sulfate was obtained from Aldrich Co., Inc. (Milwaukee, WI). Acetonitrile, UV grade and non-spectro grade, and ethyl acetate were obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI) and were used for HPLC analysis. Two hundred proof ethanol was obtained from Gold Shield Chemical Company (Hayward, CA). Distilled water for use with HPLC was deionized using a Nanopure^{R} cartridge (Sybron/Barnstead Co., Boston, MA).

Biological products. Human glycoprotein fraction VI was obtained through Miles Laboratories, Inc. (Lot #12 and 12M; Elkart, IN). Its α_1-acid glycoprotein content, determined by immunodiffusion assay (237) using M-Partigen^{TM} (Calbiochem-Behring Co., La Jolla, CA), was estimated to be 70% (w/w). Glucurase^{R}, containing β-glucuronidase and sulfatase, was obtained from Sigma Chemical Co.

Miscellaneous equipment and supplies. Dialysis cells (1 ml capacity), used for protein binding determination, were obtained from Technilabs Instruments, Inc. (Pequannocock, NJ). The dialysis membranes used (average pore size 24 Å) were from VWR Scientific, Inc. (San Francisco, CA). The equilibrium dialysis was carried out using a shaker bath (Thermomix 1480, Braun-Melsungen, AG, Federal Republic of Germany) at 37°C. The caraway capillary tube (nonheparinized) was obtained from American Hospital Supply Co. (Miami, FL) and was used for separation of serum and red blood cells. The centrifuges used in the studies were a microcentrifuge (MB centrifuge) and desk-top centrifuge (Model HN) from International Equipment Co. (Needham Heights, MA). Disposable glass tubes for sample preparation were obtained from various sources including Scientific Products, Inc. (McGaw Park, IL), VWR Scientific, Inc., and Fisher Scientific Co. The Vortex mixer (Thermolyne Maxi Mix^{TM}) was obtained from Sybron/Thermolyne Co. (Dubuque, IA). pH was measured using a pH meter (Cat. 7664) from Leeds & Northrup Co. (Philadelphia, PA) and reference buffer solution (pH 7.00) for measurement was obtained from Scientific Products, Inc. The sterile disposable filter units (0.45-μm pore size) were obtained from Millipore Co. (Bedford, MA), and were used to filter disopyramide infusion solution (Cathivex^{R}) and glycoprotein solution (Millex^{R}-HA).

II-2. Preparation of Disopyramide Injection Solutions

Purification of disopyramide. Disopyramide phosphate from Norpace^{R} capsules was dissolved in approximately 10 ml/g 1% phosphoric acid aqueous solution and then filtered. The aqueous solution was extracted with an equal volume of dichloromethane in three portions (5:3:2) after alkalinization with excess sodium hydroxide. The organic phase was subsequently extracted with an equal volume of 1% phosphoric acid in portions (5:3:2) and the aqueous solution of disopyramide was re-extracted with dichloromethane after alkalinization as in the previous step. After solvent evaporation, the disopyramide base was dissolved in a minimum volume of hot methanol and hot water was added until the disopyramide methanol/water solution was saturated. Disopyramide was then crystallized by cooling the solution.

Preparation of disopyramide injection solution. One gram of the recrystallized disopyramide base was dissolved in 20 ml of 8% phosphoric acid in water and adjusted to pH 7.4 by the addition of 5 N aqueous sodium hydroxide. The solution was diluted with a normal saline solution to yield a final solution of 5 mg/ml and filtered through a Cathivex^{R}-0.45-μm sterile filter before administration.

Separation of R- and S-disopyramide. R- and S-disopyramide were separated as described by Burke, Jr. *et al* (57) by fractional crystallization of their diastereomeric bitartrate salts. The recrystallized racemic disopyramide base was dissolved in methanol at a concentration of 1 g disopyramide base in 7 ml methanol, and one equivalent of L-(+)-tartaric acid was added. The solute was then dissolved by gentle heating

in a steam bath. The residue was removed by filtration. Five volumes of acetone were then added to each volume of methanolic solution and R-disopyramide-L-tartaric acid bitartrate was crystallized in a freezer. The needle crystals were then collected by filtration and recrystallized in 1:10 methanol/acetone five times to yield optical pure disopyramide bitartrate, $[\alpha]_D^{25} = +36.0^{\circ}$. S-disopyramide was subsequently extracted from the filtrate after evaporation of methanol/acetone and alkalinization. The enantiomeric disopyramide bitartrate, formed using D-(-)-tartaric acid, was obtained by fractional crystallization by the same procedure, $[\alpha]_D = -36.0^{\circ}$.

The R- and S-disopyramide obtained were dissolved in normal saline solution prior to use. The solution was also filtered through a Cathivex^R-0.45-μm sterile filter during drug infusion.

II-3. Experimental Rabbit Preparation

Male New Zealand white rabbits ranging from 2.1 to 3.2 kg were obtained from Nitabell Co. (Hayward, CA). Before the rabbit was placed in a rabbit restraining cage (Plaslabs, Lansing, MI) for the study period, its bladder was cannulated with a pediatric Foley catheter (Bardex^{R}, 8 Fr, 3-cc balloon; C.R. Bard, Inc., Murray Hill, NJ) through the urethra when necessary. The marginal ear veins in both ears were cannulated with polyethylene tubes with an Intracath^{R} (22 Ga, 8 in) which was obtained from Deseret Co. (Sandy, UT). To prevent clotting during the study, the catheters were filled with normal saline solution containing 10 U/ml heparin sodium.

In most of the studies, disopyramide was constantly infused into a marginal ear vein using an infusion pump (Model 975, Harvard Apparatus Co., Inc., Millis, MA), equipped with a 20-ml disposable syringe (Monoject/Sherwood, Brunswick Co., St. Louis, MO), connected to the Intracath^{R} by a plastic extension tube (Pharmaseal, Inc., Toa Alta, Puerto Rico). Another Harvard infusion/withdrawal pump (Model 931) attached with a 5-cc exchangeable glass syringe (Multifit, Becton, Dickinson and Co., Rutherford, NJ) was connected to still another Intracath^{R} in the rabbit's marginal ear vein in the other ear using extension tubes when a continuous blood withdrawal was being carried out.

II-4. Electrocardiographic Response Measurement

The increase of QRS duration in the electrocardiogram in the rabbit was used as a measure of the pharmacological response to disopyramide. Three needle electrodes (Hewlett-Packard, Inc., Palo Alto, CA) were placed subcutaneously on the rabbit back and neck and connected to an electrocardiograph (Model 1500B, Hewlett-Packard, Inc.). The electrical signals were displayed on an oscilloscope (Model 5115, Tektronix, Inc., Beaverton, OR) and were stored on an FM tape recorder (Model 4DS, Lockheed, Inc., Plainfield, NJ). The stored signal was subsequently played back and expanded on the storage oscilloscope which was equipped with a differential amplifier (Model 5A22N) and a time base (Model 5B10N). For each time point, 10 measurements were randomly sampled and averaged.

II-5. Disopyramide Assay

Disopyramide concentration in serum, buffer, urine, or injection solution was determined using a specific reverse-phase high-pressure liquid chromatographic (HPLC) method. To a 100-µl unknown or blank sample solution, the following were added: 100 µl of 10 mg/ℓ chlorodisopyramide (internal standard) in water, 100 µl water for the unknown sample solution or 100 µl disopyramide standard water solution for the blank sample, and 100 µl of 5 N sodium hydroxide. The mixture was subsequently extracted with 5 ml ethyl acetate. Four milliliters of the ethyl acetate was then extracted with 175 µl of 1% phosphoric acid solution. One hundred microliters of the aqueous extract was sampled by a microsyringe (Hamilton Co., Reno, NV) and injected into an HPLC system.

The HPLC equipment used for the disopyramide assay consisted of the following: a manual loop sample injector (Model 7120, Rheodyne, Inc., Cotati, CA), an Ultrasphere-octyl column (Beckman, Inc., Berkeley, CA) of 25 cm length and 4.6 mm bore, a high-pressure liquid chromatographic pump (Beckman, Model 110A), a fixed wavelength UV detector (Beckman, Model 153), a recorder (Omniscribe^{R} B-5000, Houston Instrument Co., Austin, TX), and stainless steel tubes and adaptors (Upchurch Scientific, Inc., Oak Harbor, WA) connecting various components of the system.

The injected sample was eluted with a mobile phase containing water, acetonitrile (UV grade), phosphoric acid, and 5 N sodium hydroxide in the ratio of 50:50:0.1:0.1 (v/v), at a flow rate of 1.5 ml/min. Detection was carried out at 254 nm. Standard curves were prepared each time biological samples were assayed. All samples were assayed within 3 days after collection.

Disopyramide content in whole blood samples was assayed with a slight modification of the extraction procedure. To a 400-µl blood sample, 400 µl of 10 µg/ml chlorodisopyramide in acetonitrile (UV grade) was added and vortexed. The solution was then decanted into another test tube and 400 µl was sampled for extraction. To the water/acetonitrile mixture solution, 200 µl of 5 N sodium hydroxide and 5 ml ethyl acetate was added and vortexed. Four milliliters of the ethyl acetate were then extracted with 250 µl of 1% phosphoric acid solution. A 100-µl aqueous extract was then sampled and assayed as described above. Standard curves were obtained by adding disopyramide to blank blood and prepared as described above.

Using the chromatographic conditions described, disopyramide and chlorodisopyramide had retention times of 8 and 11 min, respectively. No interference of endogenous compounds was observed in the rabbit serum collected before the disopyramide infusion. No metabolite was detected in rabbit serum prior to enzyme hydrolysis. After Glucurase^{R} incubation, an unknown metabolite was found to have a retention time of 4.5 min, whereas N-monodealkylated disopyramide had a retention time of 5 min. The lower quantitation limit of disopyramide, defined as peak-to-noise ratio of 7:1, was 10 ng in the preextracted samples at optimum chromatographic conditions. The coefficient of variation of the assay for samples containing 100 ng to 1 µg in 100 µl was less than 4%.

II-6. Indocyanine Green Assay

Indocyanine green in serum was assayed using a specific reverse-phase high-pressure liquid chromatographic method. To a 100- or 200-µl sample, an equal volume of acetonitrile (nonspectro grade) was added. After vortexing, the mixture was placed in nonheparinized Caraway tubes and centrifuged at 7900 x g for 2 min. The supernatant was then placed in an automatic injector (WISP^{R} 710B, Waters Associates, Inc., Milford, MA) and 50 µl was injected onto an Alltech C18 10-µm column (25 cm length and 4.6 mm bore, Alltech Associates, Deerfield, IL), connected to a high-pressure liquid chromatographic pump. The sample was eluted with a mobile phase containing water, acetonitrile (nonspectro grade), tetrabutylammonium hydrogen sulfate, 85% phosphoric acid, and 5 N sodium hydroxide in the ratio of 50:50:0.05:0.05:0.12 at a flow rate of 1 ml/min. Detection was carried out at 800 nm using a variable wavelength detector (Model 100-30, Hitachi, Ltd., Tokyo, Japan) which was modified for HPLC usage by Beckman, Inc. The peak height was compared with that of standard solutions in serum. All injections were made in duplicate.

Using the chromatographic conditions described above, indocyanine green had a retention time of 6 min. An unidentified metabolite or degradation product was seen with a retention time of 3.5 min. The lower quantitation limit of the assay, defined as a 7:1 peak-to-noise ratio, using a new column, was 20 ng/ml when 50 µl was injected. After approximately 100 injections, the peaks broadened and the sensitivity decreased to 50 ng/ml. A freshly prepared standard solution was injected for every 5 injections of samples to ensure stable column conditions.

II-7. Protein Binding Determination

Protein binding was determined by equilibrium dialysis. A volume of 800 µl serum was dialyzed against 800 µl of 0.13 M phosphate buffer, pH 7.4, in 1 ml dialysis cells in a shaker bath at 37°C for 8 hr. The dialysis membrane was pretreated by a fresh soaking in water for 10 min, in ethanol for 15 min, and then in 0.13 M phosphate buffer (pH 7.4) for 60 min before usage. The serum and buffer side after dialysis were assayed for disopyramide content as described in Section II-5. After 8 hr dialysis, there is an increase of approximately 10% in the volume of serum and a corresponding decrease in buffer volume. The unbound fraction was calculated as the ratio of buffer concentration to serum concentration without correction for water flux.

Chapter III.

Comparison of Electrocardiographic Response and Disposition of R-Disopyramide and S-Disopyramide

Synthetic drugs are often composed of recemic mixtures of optical isomers. Optical isomers have exactly the same physical and chemical properties except for their optical rotations, which are the same in magnitude but opposite in direction. Their interaction with biological macromolecules such as receptors, enzymes, or transport proteins is, however, often stereoselective. The association of enantiomers with stereospecific macromolecules results in diastereoisomers, which are different in physical and chemical properties. When drug-receptor binding is stereoselective, enantiomers induce pharmacological response to different extents, because their drug-receptor binding complexes have different affinities or association/dissociation rates. If binding to either hepatic enzymes, active transport proteins, or serum binding proteins is stereospecific, the disposition of the enantiomers will differ.

The cardiac electrophysiological effect of R- and S-disopyramide has been compared in canine Purkinje fibers (258) and rabbit ventricles (57). R- and S-disopyramide were found to affect repolarization differently, while they had a similar effect on depolarization. When anticholinergic effect was compared, S-disopyramide was about 3- to 4-fold more potent than R-disopyramide (128,256). The comparison of electrocardiographic response of R- and S-disopyramide *in vivo* has not been reported.

Disposition of R- and S-disopyramide has been compared in dogs (129). The clearance of S-disopyramide was significantly greater

than that of R-disopyramide. The steady-state volumes of distribution of the two compounds were similar, resulting in a longer half-life for R-disopyramide than for S-disopyramide. The difference in disposition between R- and S-disopyramide has not been reported in any other species.

In this chapter, electrocardiographic response, clearance, and serum protein binding of R- and S-disopyramide are compared in rabbits. The data obtained are used to design experiments for studying the influence of serum protein binding on pharmacological response and disposition of drugs, the subject of the subsequent chapters.

EXPERIMENTAL

Animal experiments. Seventeen New Zealand white rabbits weighing 2.1 to 2.7 kg were studied. Nine of them were used to study S-disopyramide, the other eight for R-disopyramide. Drugs were infused through the marginal ear vein of the restrained rabbit for 100 minutes, and blood samples were taken from the marginal ear vein of the other ear during the infusion period. A loading infusion, approximately 3.8 times the maintenance infusion, was infused during the first 15 minutes to help reach steady state quickly. The maintenance infusion rates of R- and S-disopyramide are listed in Tables III-1 and -2, respectively. One milliliter of blood was sampled at 0, 4, 8, 14, and 20 minutes and 3 ml blood were collected at 40, 60, 80, and 100 minutes after the start of drug infusion. The blood was allowed to clot and two hours later the serum was separated by centrifugation. Equilibrium dialysis was used to determine the unbound fraction in serum. No urine was collected during the study. Electrocardiographic response was recorded throughout the study.

Drug binding to human glycoprotein in vitro. One milligram of human glycoprotein fraction VI was added to 1 ml of blank rabbit serum. Eight hundred microliters of 0.13 M phosphate buffer, pH 7.4, containing various concentrations of R- or S-disopyramide bitartrate, 3, 6, 9, 12, 16, 20, and 24 mg/l, were dialyzed for 8 hours against 800 µl of rabbit serum containing human glycoprotein. The unbound fraction of disopyramide was calculated from the ratio of disopyramide concentration in buffer to that in

serum after dialysis.

Data analysis. The unbound fraction, total serum disopyramide concentration, and relative change of QRS duration during the period of 40 to 100 minutes were used to compare R- and S-disopyramide. The total clearance of disopyramide was calculated by:

$$Cl = \frac{R}{C_{p,ss}} \qquad \text{(Eq. III-1)}$$

where R is the maintenance infusion rate, and $C_{p,ss}$ is the averaged total serum concentration of disopyramide.

The relative change of QRS duration for R- and S-disopyramide were compared using an F-test. The pharmacological response was linearly fitted to the unbound concentration of R- and S-disopyramide. The sum of squares due to error (SSE(R)) was calculated for each enantiomer. Another line was fitted to the hybrid of R- and S-disopyramide data and another sum of squares due to error (SSE(F)) was calculated. A test statistic (F) was then calculated:

$$F = \frac{SSE(R)-SSE(F)}{2} \div \frac{SSE(F)}{n_1 + n_2 - 4} \qquad \text{(Eq. III-2)}$$

where n_1 and n_2 are numbers of data points for R- and S-disopyramide, respectively.

The binding of R- and S-disopyramide to human glycoprotein fraction VI spiked in the rabbit serum was compared assuming only one class of binding sites. Equation I-22 was rearranged to the following relationship between the unbound fraction and unbound

drug concentration:

$$\frac{1}{1/\alpha - 1} = \frac{Kd}{Pt} + \frac{Cu}{Pt} \quad . \qquad \text{(Eq. III-3)}$$

The parameters of Eq. III-3 were then obtained by fitting the binding data for both R- and S-disopyramide, and Eq. III-2 was used to calculate the statistic, F, to decide whether the two straight lines were the same or not.

All fitting procedures were conducted using the NEWLAB program in the PROPHET system.

RESULTS

The average unbound fraction of R- and S-disopyramide at steady state in each rabbit is listed in Table III-1 and III-2, respectively. The unbound fraction averaged 0.84 for R-disopyramide, and 0.86 for S-disopyramide. There is no significant difference between the two enantiomers with respect to binding to rabbit serum proteins (non-paired student t-test, $P>0.1$).

The pharmacological responses to R- and S-disopyramide, in terms of relative change of QRS duration, are also listed in Table III-1 and III-2, and compared in Fig. III-1. A linear model was used to fit the relationship between QRS duration change and unbound disopyramide concentration at steady state. The effect of R-disopyramide and S-disopyramide was found to be significantly different ($F=11.23$, $P<0.01$). The intercepts of both fitted lines are not significantly different from the origin ($P>0.05$).

Total body clearances of R-disopyramide and S-disopyramide are listed in Tables III-1 and -2, respectively. They are compared in Fig. III-2. A significant correlation was found between S-disopyramide clearance and the drug concentration (Spearman's $r=-0.917$, $P=0.001$). A more than two-fold difference in clearance in the measured concentration range is seen. There is also a small but significant trend of decreasing R-disopyramide clearance with increasing drug concentration in serum. (Spearman's $r=-0.81$, $P=0.011$).

Table III-1. EXPERIMENTAL RESULTS OF PROTEIN BINDING, CLEARANCE, AND PHARMACOLOGIC ACTION OF R-DISOPYRAMIDE IN 8 RABBITS DURING STEADY-STATE DRUG INFUSION

Rabbit (#)	Infusion Rate (μg/min/kg)	$C_{p,ss}$ (mg/ℓ)	Unbound Fraction	$C_{u,ss}$ (mg/ℓ)	Δ QRS (%)	Clearance (ml/min/kg)
110981	103.6	3.1	0.77	2.4	10.2	33.4
111381	94.8	2.8	0.89	2.5	9.4	33.9
112581	219.3	6.7	0.84	5.6	30.9	32.7
120281	302.3	9.3	0.85	7.9	48.7	32.5
121981	119.0	3.7	0.84	3.1	16.3	32.2
011182	219.3	8.1	0.83	6.7	32.8	27.1
011782	215.0	6.9	0.84	5.8	30.7	31.2
011982	343.8	13.7	0.85	11.6	55.3	25.1

Table III-2. EXPERIMENTAL RESULTS OF PROTEIN BINDING, CLEARANCE, AND PHARMACOLOGICAL ACTION OF S-DISOPYRAMIDE IN 9 RABBITS DURING STEADY-STATE DRUG INFUSION

Rabbit (#)	Infusion Rate (μg/min/kg)	$C_{p,ss}$ (mg/ℓ)	Unbound Fraction	$C_{u,ss}$ (mg/ℓ)	ΔQRS (%)	Clearance (ml/min/kg)
120481	219.3	3.9	0.85	3.3	19.8	56.2
121181	290.9	6.6	0.86	5.7	21.8	44.1
121781	436.2	15.6	0.81	12.7	44.9	28.0
123081	219.3	3.6	0.86	3.1	12.3	60.9
011382	343.8	8.9	0.87	7.7	28.0	38.6
011482	360.2	10.8	0.88	9.5	28.0	33.4
012182	238.3	6.9	0.88	6.1	19.7	34.5
020182	166.8	3.3	0.82	2.7	15.3	50.5
020282	296.6	6.6	0.88	5.8	31.7	44.9

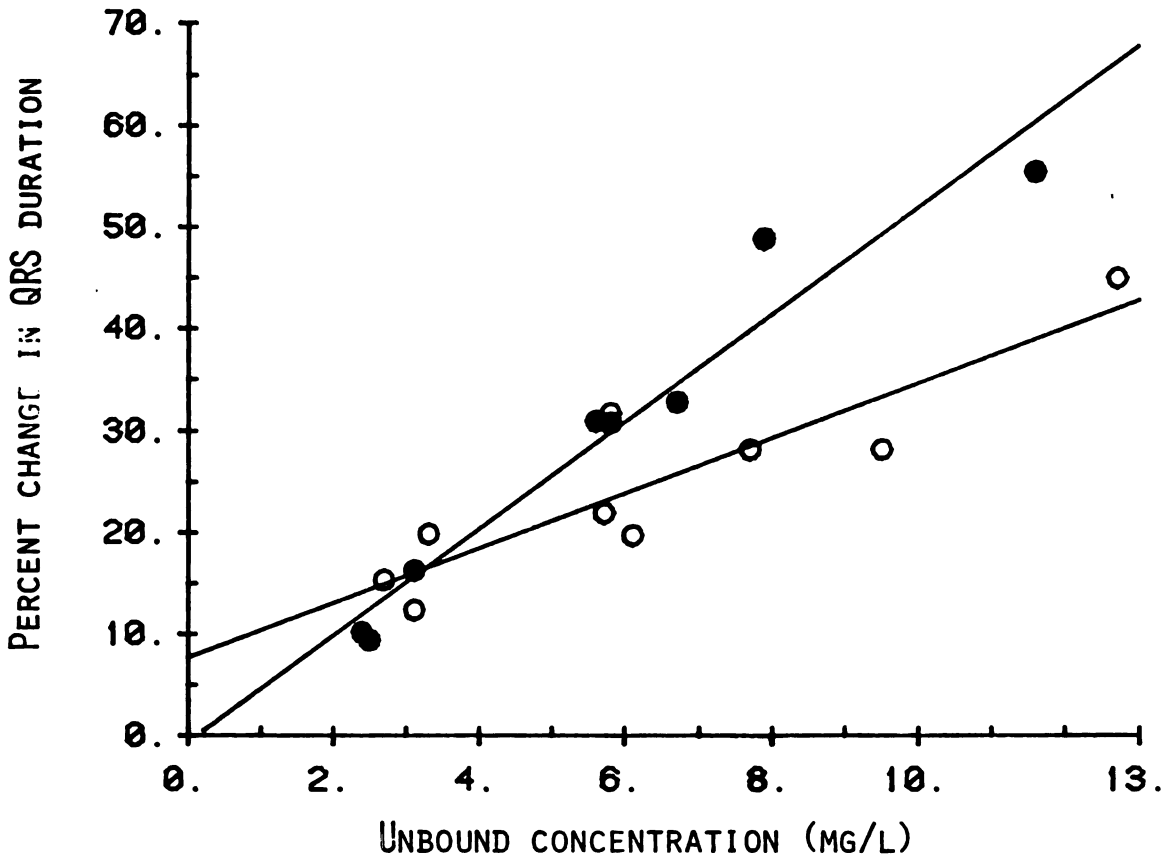


Fig. III-1. Comparison of pharmacological activity of R-disopyramide and S-disopyramide in changing QRS duration of the EKG with steady-state infusion of drugs. (●), R-disopyramide; (○), S-disopyramide. The two fitted lines are statistically significantly different ($F = 11.23$; $P < 0.01$).

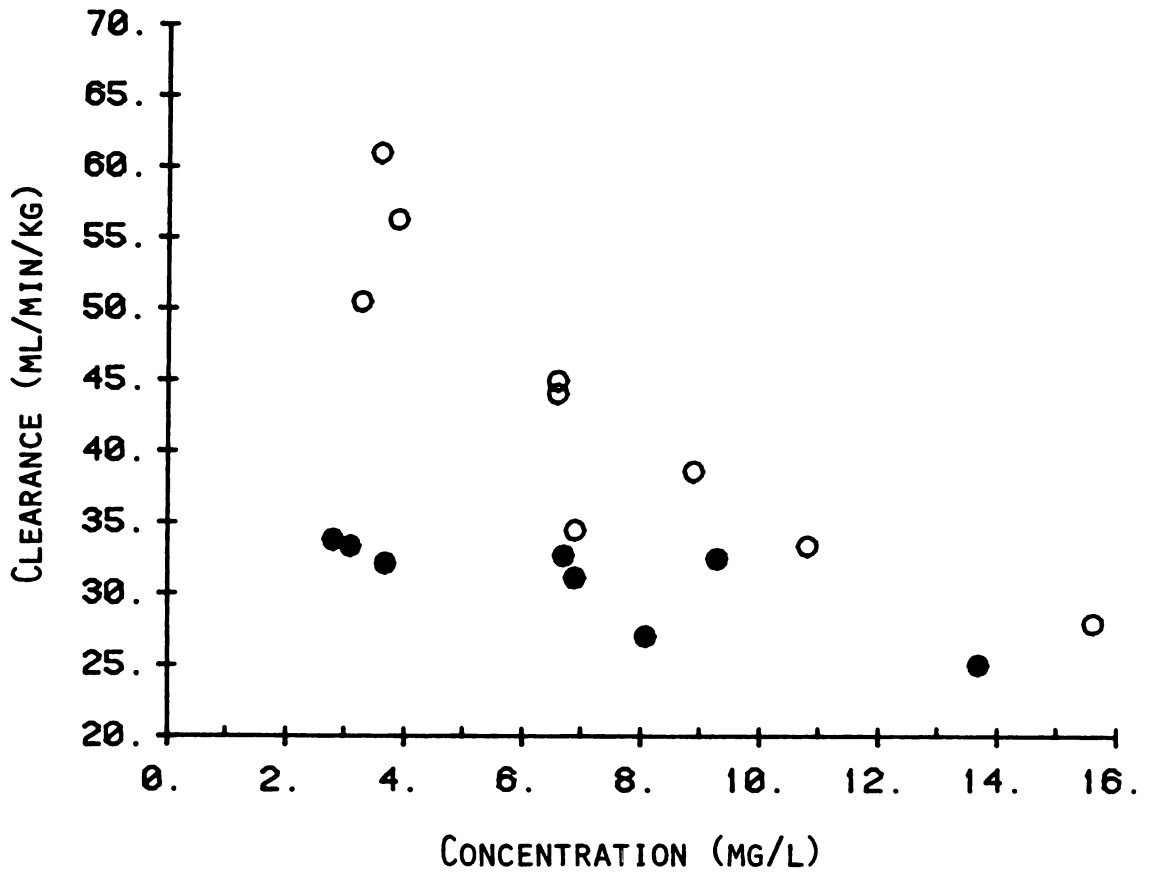


Fig. III-2. Concentration dependence of total body clearance of R-disopyramide (●) and S-disopyramide (○) in rabbits. There is a significant negative correlation between steady-state concentration of total drug in serum and clearance of both R-disopyramide ($r = -0.81$; $P = 0.011$) and S-disopyramide ($r = -0.917$; $P = 0.001$).

The binding of R- and S-disopyramide to human glycoprotein fraction VI is compared in Fig. III-3. The dissociation equilibrium constant (Kd) and binding capacity (Pt) were 4.2 μM and 9.0 μM respectively for R-disopyramide, 4.7 μM and 9.7 μM respectively for S-disopyramide, which resulted from computer fitting using Eq. III-3. The two fitted lines of R- and S-disopyramide binding data were not significantly different from each other ($F=-0.14$, $P>0.5$).

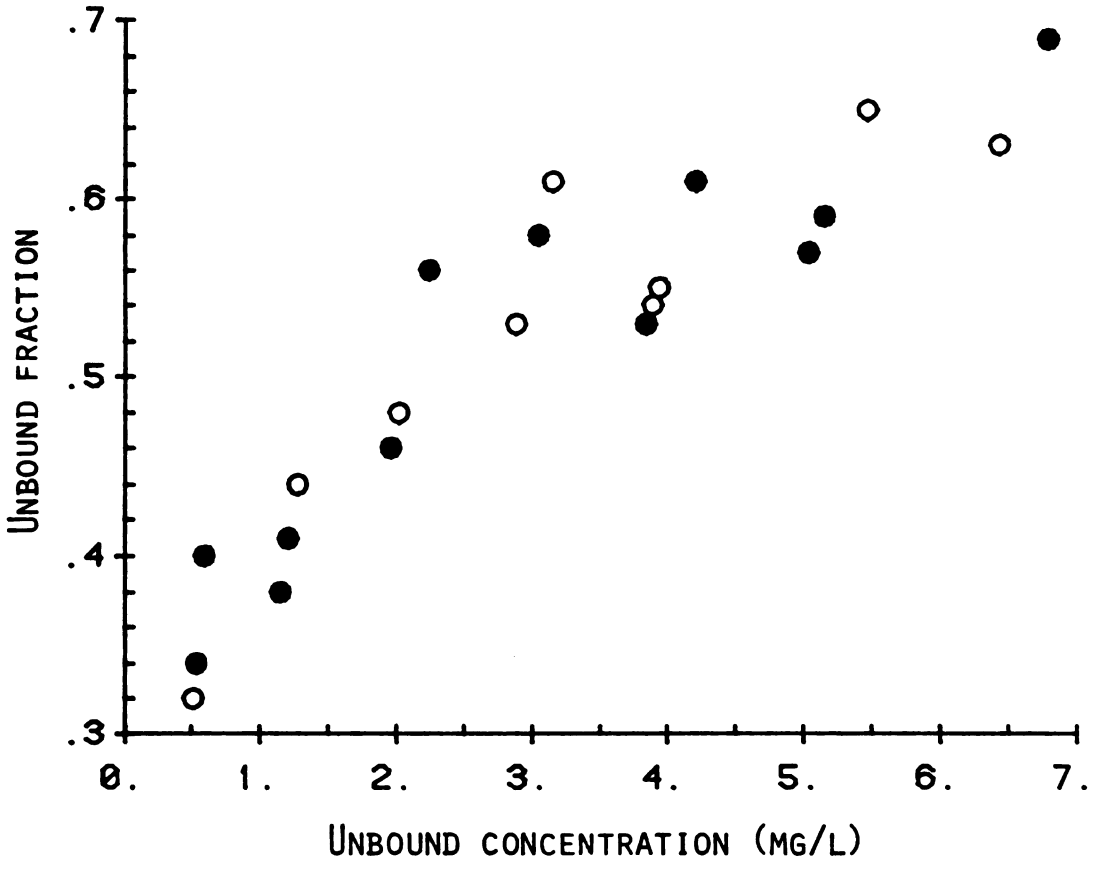


Fig. III-3. Unbound fraction of R-disopyramide (●) and S-disopyramide (○) at various concentrations in rabbit serum spiked with human glycoprotein fraction VI. The relationships for the two enantiomers are not significantly different ($F = -0.14$; $P > 0.5$).

DISCUSSION

To test whether unbound drug concentration or total drug concentration in serum reflects the changes in the pharmacological response when serum binding is altered, it is desirable to select a model drug for which drug binding can be significantly altered and pharmacological response can be easily quantitated. Because R-disopyramide binds to rabbit serum protein and human glycoprotein fraction VI to the same extent as S-disopyramide does, there will be no difference in serum protein binding between the two enantiomers before and after human glycoprotein fraction VI treatment in rabbits. However, because R-disopyramide is more potent in changing QRS duration at the working concentration range, it was considered to be the preferred model drug to test the hypothesis.

Current pharmacokinetic models predict different steady-state drug concentrations for a given alteration of serum protein binding for drugs with low and high clearance (Section I-1-b). For drugs with low clearance, an increase in serum binding does not affect steady-state concentration of unbound drug but does increase the total steady-state concentration because unbound clearance is constant (Eq. I-12) and total clearance is decreased (Eq. I-11). On the other hand, for drug with a clearance which is close to the blood flow of the eliminating organ, the steady-state concentration of unbound drug decreases with increased binding, whereas the total drug concentration in blood stays unchanged. Considering that R-disopyramide clearance is always lower than that of S-disopyramide

and that non-renal clearance accounts for most of drug elimination, it can be predicted that total R-disopyramide concentration in serum can be increased more than that of S-disopyramide at the same extent of binding alteration. To demonstrate that bound drug concentration is not related to pharmacological response, R-disopyramide is therefore the preferred model drug.

To demonstrate directly that it is the unbound drug concentration and not the total drug concentration in serum that is related to the pharmacological response of drugs when the binding changes, it is necessary to select a test drug where the unbound steady-state concentration is changed by the binding alteration. S-Disopyramide, which has a high clearance (close to blood flow) at low drug concentration in rabbits, can possibly be used for the purpose if the alteration in pharmacological response can be quantitated. It is technically difficult to measure small changes in QRS duration. There is a substantial variation in the baseline of the QRS duration, which limits the usefulness of S-disopyramide for these studies. From data shown in Figs. III-1 and III-2, a steady-state concentration of S-disopyramide of approximately 4 mg/ℓ before human glycoprotein treatment is considered optimal: If a substantial change in the unbound concentration, such as changing it from 4 mg/ℓ to 2 mg/ℓ, can be achieved by glycoprotein treatment, the decrease of the percent QRS duration change, from 18% to 12%, may be sufficient to be statistically significant. In addition, at these concentrations, S-disopyramide will act as a high extraction ratio drug.

Because the influence of serum protein binding on the hepatic elimination of a high extraction ratio drug is especially of interest, S-disopyramide was selected as a model drug. Because of concentration-dependent serum protein binding in rabbits after glycoprotein treatment, as shown in Fig. III-3, and concentration-dependent clearance, as shown in Fig. III-2, it would be necessary to carry out the study at the lowest possible S-disopyramide concentration.

S-disopyramide and R-disopyramide appear to bind differently to human serum proteins (personal communication, K.M. Giacomini). The differences, however, were not found in this study, which used human glycoprotein fraction VI in rabbit serum. Several possible explanations can be postulated to account for the discrepancy: 1) the human glycoprotein fraction VI which was obtained through Miles Laboratories might actually have been partially denatured during the preparation process, with an alteration of the stereoselective binding sites; 2) α_1-acid glycoprotein does not contain stereoselective binding sites for disopyramide. Other proteins present in human plasma might binding disopyramide and actually be responsible for the observed stereoselective binding seen by Giacomini; 3) the stereoselective binding is inhibited or masked by components of rabbit serum.

The binding capacity of R-disopyramide and S-disopyramide to human glycoprotein fraction VI determined in this study was much lower than the molarity of α_1-acid glycoprotein determined by immunodiffusion assay. The determined concentration of α_1-acid glycoprotein in the

spiked rabbit serum was 17 μM before dialysis, it should be approximately 15 μM after correction for water flux, whereas the binding capacity was 9.0 μM for R-disopyramide and 9.7 μM for S-disopyramide. It has been reported that immunodiffusion assay does not differentiate between native and desialyzed α_1-acid glycoprotein (34). The immunodiffusion assay also may not differentiate between native and denatured α_1-acid glycoprotein which does not bind disopyramide.

The QRS duration change was used as a measure of the pharmacological response in this study as well as in studies presented in Chapters IV and V. One of the effects of disopyramide and other Class I antiarrhythmic drugs is to decrease the conduction velocity by blocking fast sodium channels, reflected in an increase in the QRS duration (Section I-5-c). Although QT_c interval has been argued to be more representative of the antiarrhythmic effect of Class I agents and QRS duration is considered to indicate toxicity (28), the QRS duration has been shown to be better correlated with the concentration of Class I antiarrhythmic drugs than the QT_c interval (158). A good correlation between QRS duration and antiarrhythmic effect has been demonstrated (252). In this study, the QRS duration change is shown to be well correlated with R- and S-disopyramide concentrations and with RS-disopyramide as shown in Fig. IV-5, while QT_c showed a more variable change at the tested disopyramide concentration range (data not shown).

Chapter IV.

Effect of Altered Binding on Concentration-Response Relationship of RS-Disopyramide

Determining dose-response relationships have been a major interest in pharmacological studies. These relationships not only can be used to determine the potency and toxicity of drugs, but also help in understanding the mechanism of drug action. With increasing knowledge of drug disposition and development of receptor theories (14,291), it is now generally believed that a *concentration-response* relationship is more meaningful than a *dose-response* relationship, because it avoids the inter- and intrasubject variability in drug absorption and disposition.

It is generally assumed that the unbound drug concentration in serum is equivalent to the unbound drug concentration at the site of drug action, and that unbound drug concentration determines the magnitude of pharmacological action. Based on this concept, it can be postulated that the concentration-response relationship using unbound steady-state concentration is independent of the extent of serum protein binding, whereas a concentration-response relationship using total (bound and unbound) drug concentration varies with the alteration in serum protein binding. In this study, the concentration-response relationship using both total and unbound serum concentration in two groups of rabbits with different serum protein binding are compared. The comparison is made to demonstrate that unbound drug concentration, instead of total drug concentration in serum, should be used to generate the concentration-response relationship when serum protein binding is variable.

EXPERIMENTAL

Injection of human glycoprotein fraction VI to control rabbits. Two male New Zealand rabbits (2.5 and 2.8 kg) were injected with 40 mg/kg human glycoprotein fraction VI in 5 ml sterile water. Two-ml samples of rabbit blood were sampled at 0, 2, 5, 10, 15, 25, 40, 60, 90, 120, 180, and 240 minutes and the sera were separated from the blood. Human α_1-acid glycoprotein content in the rabbit sera was determined by an immunodiffusion assay (237) using M-Partigen™. Eight hundred µl of rabbit serum from each sample was dialyzed against 800 µl of 0.13 M phosphate buffer, pH 7.4, containing 3.1 mg/ℓ of disopyramide. EKG response was recorded throughout the study.

Concentration-response studies. Ten male New Zealand white rabbits (2.4 to 3.2 kg) were randomly divided into two groups. One group was used without pretreatment. The other group was subjected to an injection of 40 mg/kg glycoprotein fraction VI in 5 ml sterile water 25 minutes before the disopyramide administration.

RS-Disopyramide solution was infused at various rates to achieve multiple steady-state concentrations in each individual rabbit using a precalibrated pump (Harvard Model 975). To quickly achieve and maintain a desired steady-state level of disopyramide, a 15-minute infusion was followed by an 85-minute maintenance infusion. When a reduction in the steady-state level was desired, the infusion was stopped for 15 minutes before the 85-minute maintenance infusion was given. The infusion rate was adjusted in order to achieve a

general ratio of drug concentration at steady state of approximately 1:2:4:3. The infusion pattern as well as the resulting disopyramide concentration and the EKG response are shown in Fig. IV-1.

Rabbit blood was sampled every 20 minutes and ΔQRS duration was measured every 10 minutes throughout the study. Serum protein binding was measured in each serum sample by equilibrium dialysis. Serum concentration of disopyramide, the unbound fraction, and the pharmacological response at each steady state were determined using data in the last 60 minutes of the maintenance period.

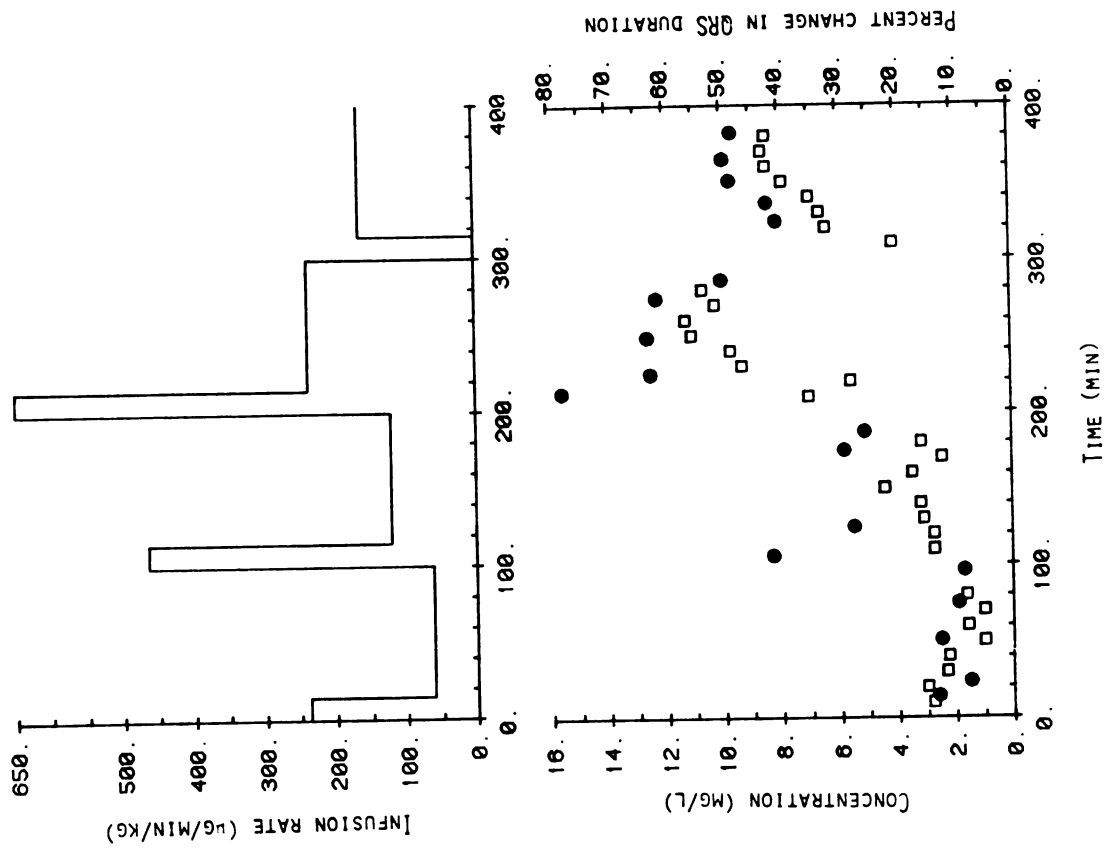


Fig. IV-1. Infusion rate, total disopyramide concentration in serum, and QRS duration change in a representative control rabbit. *Upper panel:* The infusion rate at various times. *Lower panel:* Disopyramide concentration in serum (●) with time and QRS duration changes (□), following various rates of infusion.

RESULTS

QRS duration was not changed by injection of 40 mg/kg human glycoprotein fraction VI in the two control rabbits to which no disopyramide was administered (Fig. IV-2). The QRS duration before disopyramide administration was not statistically different between the treated and untreated groups of animals in the concentration-response studies (26.9±2.6 ms and 27.6±3.1 ms, respectively; t=0.387, P>0.79). Neither was there a statistically significant difference seen in the treated animals before and after injection of 40 mg/kg human glycoprotein fraction VI prior to disopyramide adminstration (27.0±2.5 ms *vs* 26.9±2.6 ms, respectively; paired t=0.062, P>0.95).

After human glycoprotein injection, human α_1-acid glycoprotein concentration in the rabbit serum decreased rapidly in the first hour and stayed relatively constant for the rest of the study period (Fig. IV-3). Disopyramide binding was dramatically increased by the injection of human glycoprotein and decreased slightly as human α_1-acid glycoprotein concentration decreased with time (Fig. IV-3). An apparent steady-state disopyramide concentration and an increase in QRS duration were observed 30 minutes after each steady-state transition started, as shown in Fig. IV-1.

In the untreated group, the average unbound fraction was 0.83± 0.05, ranging from 0.77 to 0.97, whereas the treated rabbits had unbound fractions that ranged from 0.33 to 0.73, depending on the disopyramide concentration, as shown in Fig. IV-4.

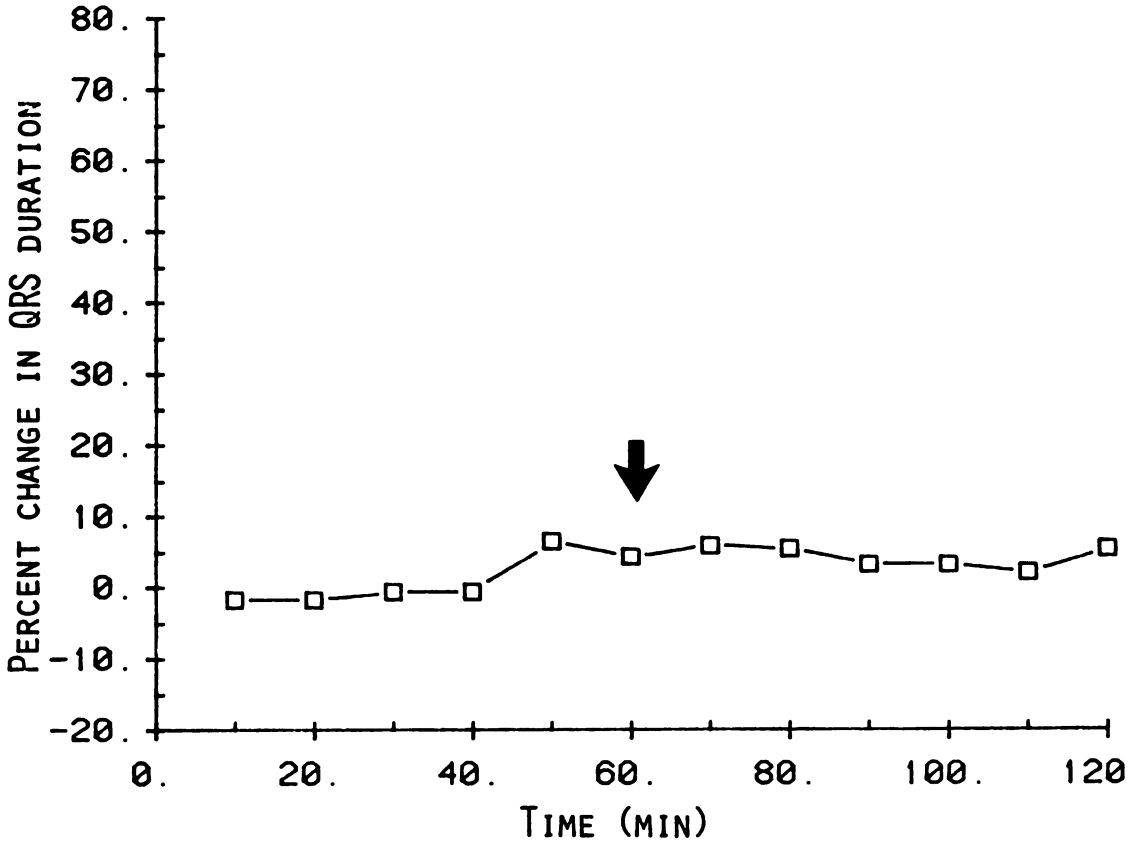


Fig. IV-2. EKG response in the rabbit (□) was unchanged by injection of 40 mg/kg human glycoprotein fraction VI (↓) in the absence of disopyramide.

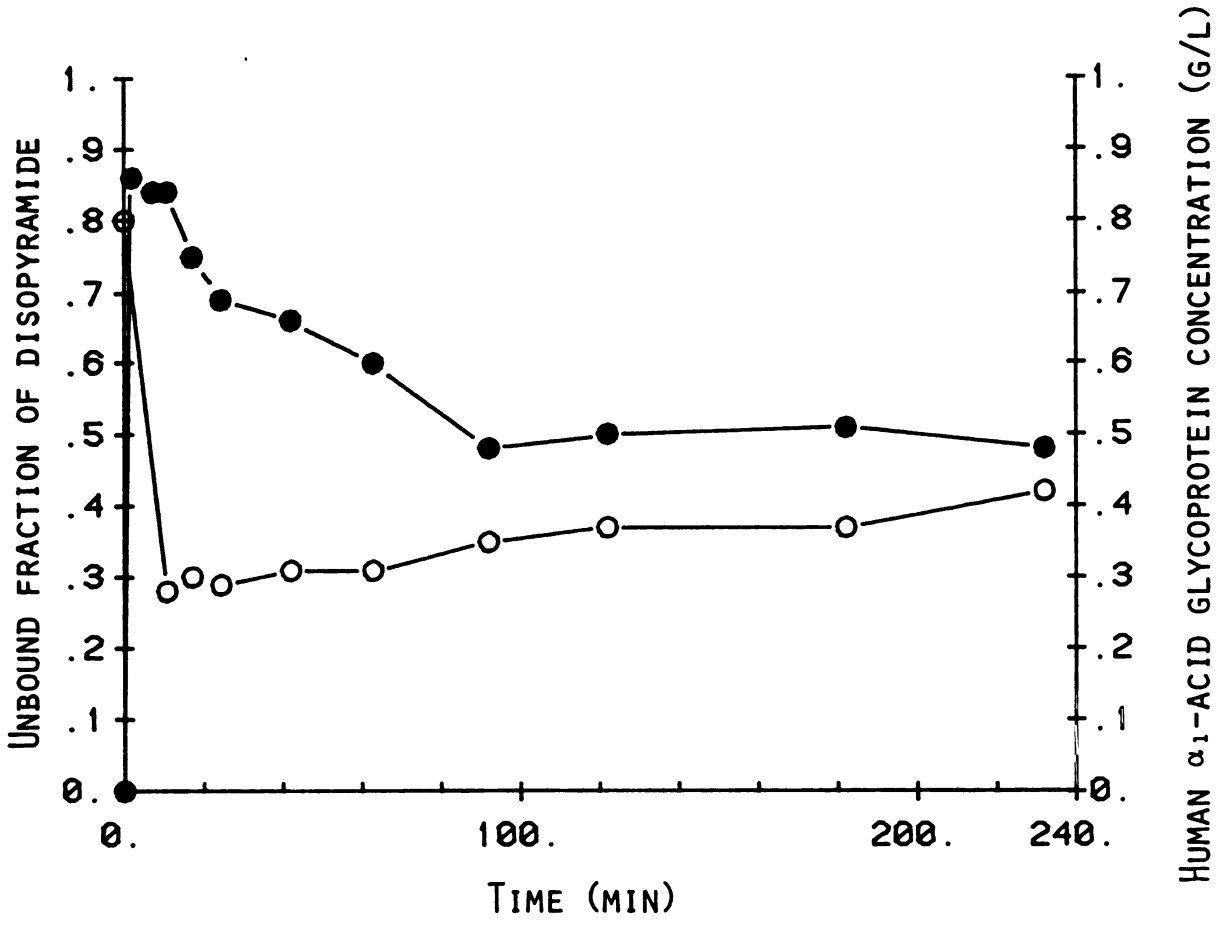


Fig. IV-3. Human α_1-acid glycoprotein concentration (●) in serum of rabbits injected with 40 mg/kg human glycoprotein fraction VI. Disopyramide unbound fraction (○) was determined *in vitro* by spiking the serum with disopyramide to a concentration of 3.1 µg/ml.

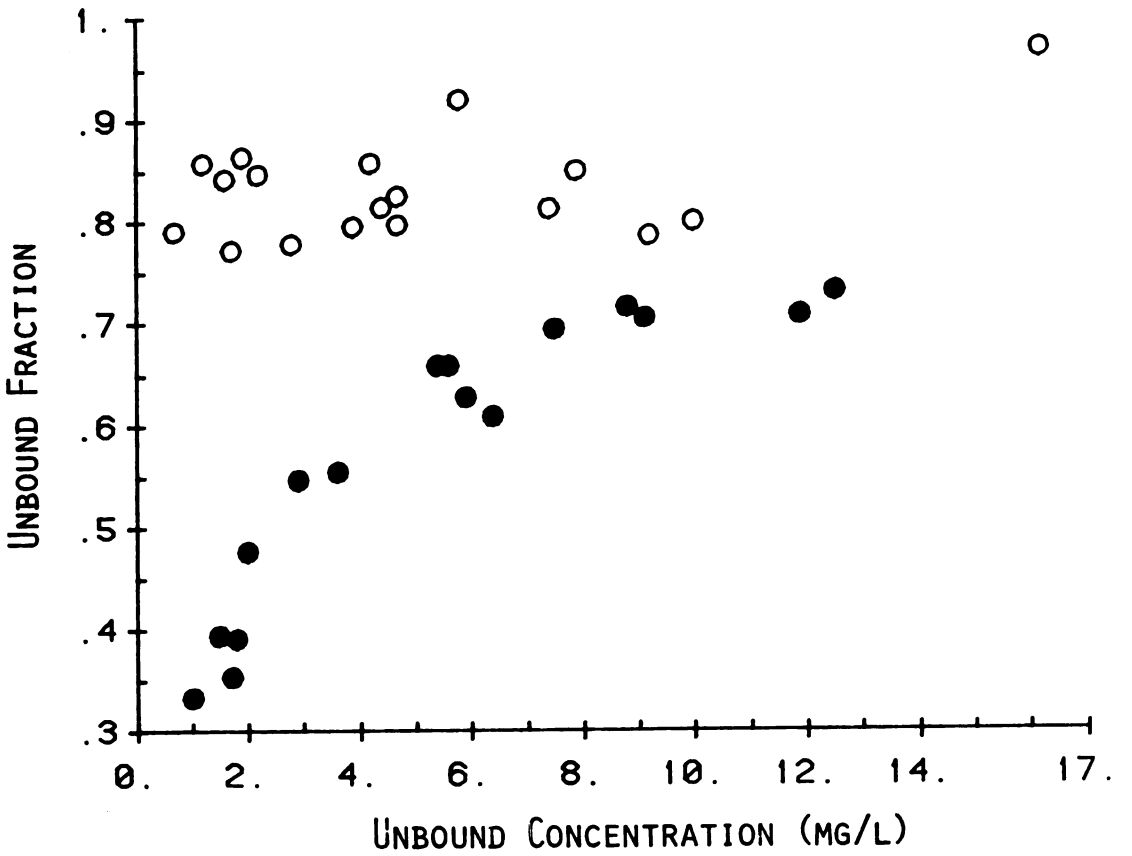


Fig. IV-4. In the untreated group (o), the unbound fraction of disopyramide in rabbit serum is relatively constant. In the rabbits treated with human glycoprotein (•), the unbound fraction of disopyramide is concentration-dependent.

The pharmacological response to disopyramide, measured as the relative change in the QRS duration, was well correlated with both the unbound and total drug concentration in serum in both groups (Figs. IV-5 and IV-6). The relationships of total serum concentration and the QRS duration lengthening in the untreated and treated rabbits were significantly different ($F=11.85$, $P<0.001$; Fig. IV-6) when a linear model were fitted to the data. There was no difference in the concentration-response relationship between the two groups in terms of unbound drug concentration in serum ($F=0.81$, $P>0.5$; Fig. IV-5).

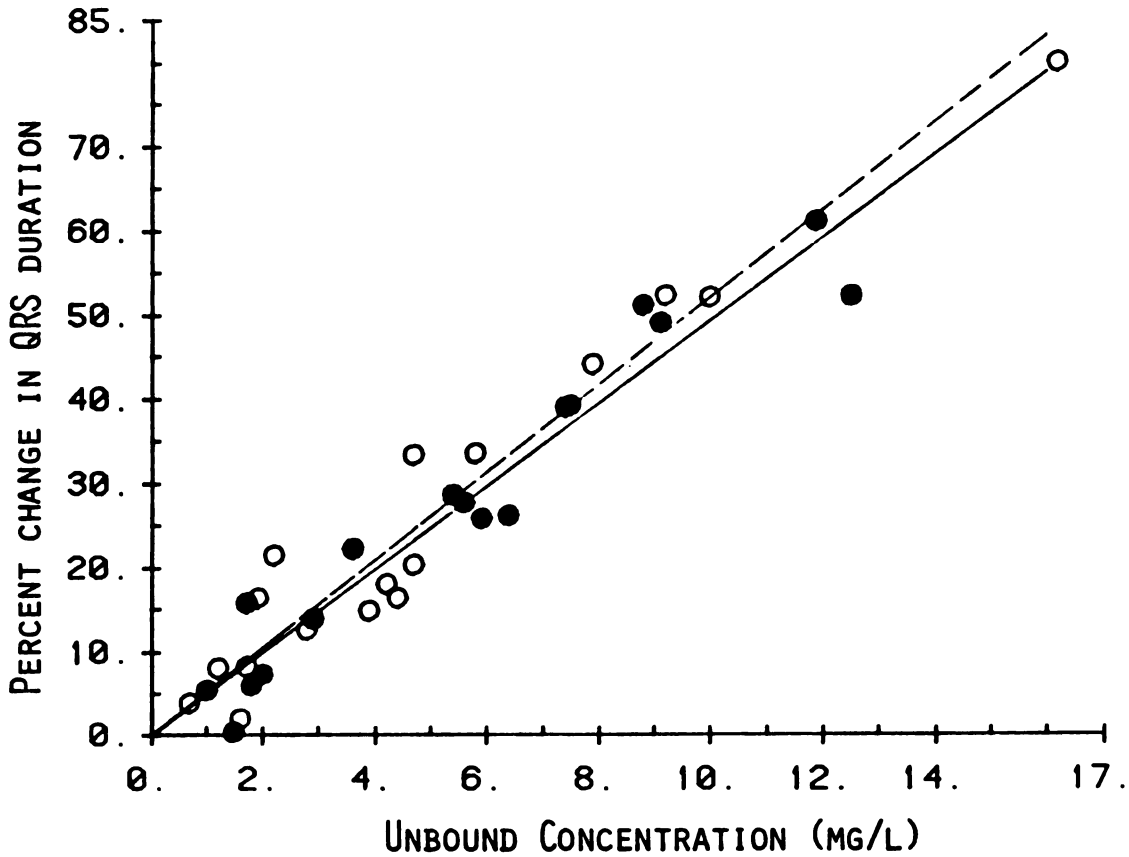


Fig. IV-5. The unbound concentration-response relationship of disopyramide is the same in rabbits given 40 mg/kg human glycoprotein fraction VI (●) as it is in control rabbits (o).

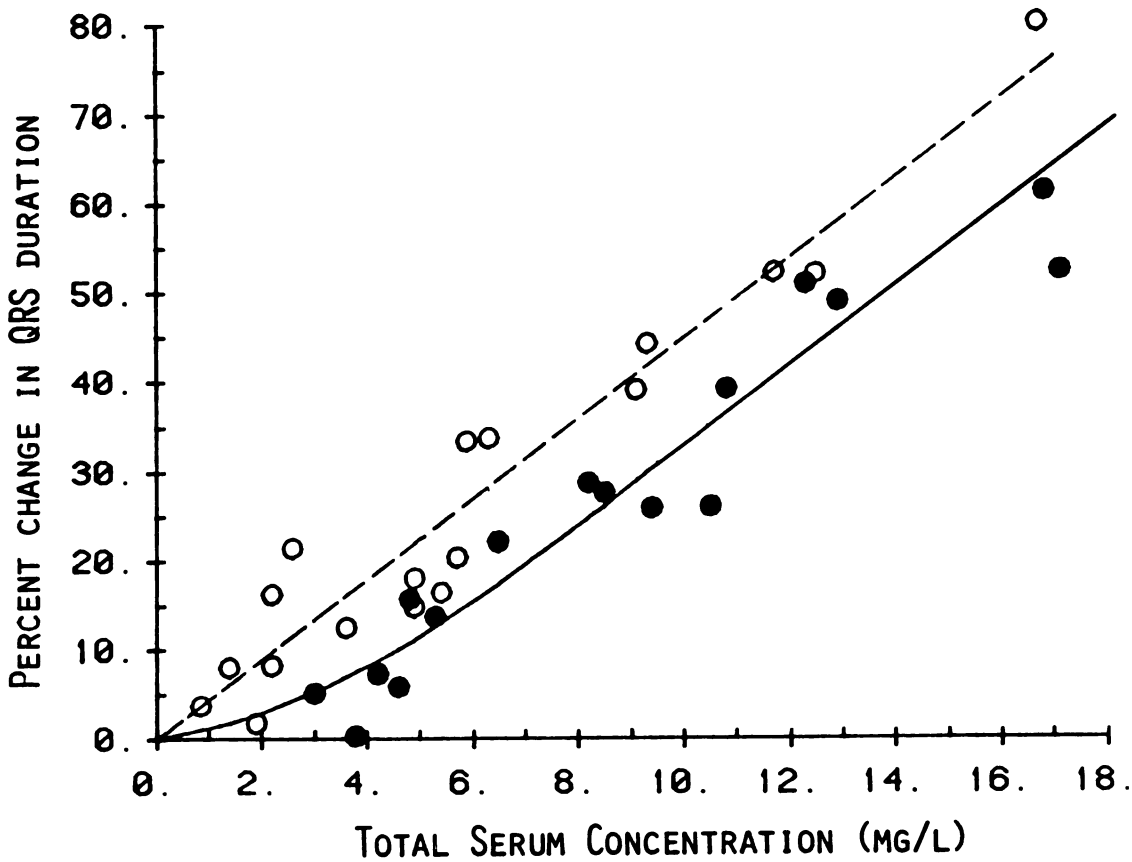


Fig. IV-6. The relationship between response and total concentration of disopyramide differs between rabbits given 40 mg/kg human glycoprotein fraction VI (●) and controls (o).

DISCUSSION

These data demonstrate that the concentration-response relationship based on unbound drug concentration is independent of drug binding in serum, whereas the relationship based on total serum concentration changes as the drug binding in serum changes. Unbound drug concentration should be used to study the concentration-response relationship under variable protein binding conditions.

When the binding increased with injection of human glycoprotein fraction VI, which contained approximately 70% α_1-acid glycoprotein, the binding was saturable, *i.e.*, decreased with increasing disopyramide concentration (Fig. IV-4). This resulted in an approximately constant concentration of bound disopyramide in the concentration range studied in the group receiving human glycoprotein fraction VI. The result of converting a constant amount of drug into an inactive form (drug bound to α_1-acid glycoprotein) should result in a parallel shift in the total concentration-response relationship to the right, which is indeed observed in Fig. IV-6. This indicates that the bound drug is unimportant for estimating the effect of disopyramide.

The concentration-response relationship was carried out first with increasing, then with decreasing infusion rates in the individual animals in order to determine whether the pharmacological response is time-dependent. As no hysteresis was found in the individual relationship, no time-dependent effect of disopyramide

was discernible.

By extrapolating the solid line in Fig. IV-6 to 0% QRS duration change, it appears that 2.6 mg/ℓ (7.7 μM) of disopyramide are bound to human α_1-acid glycoprotein when the disopyramide binding sites on the α_1-acid glycoprotein are saturated. The value is much less than the α_1-acid glycoprotein concentration (12 μM) which was determined by the immunodiffusion assay. The observed ratio of the binding capacity to the protein concentration is similar to that shown in Chapter III. The disopyramide binding capacity of the spiked human α_1-acid glycoprotein in rabbit serum is approximately 60% of the measured molarity of the α_1-acid glycoprotein. In addition to a relatively constant level of human α_1-acid glycoprotein in the rabbit serum (Fig. IV-3), the similarity of the ratios *in vivo* and *in vitro* indicates that the disopyramide binding capacity in the treated rabbit serum is essentially a constant.

It has been reported that a serum disopyramide concentration does not represent the pharmacological action when the drug is rapidly distributing from serum into the rest of body after an intravenous dose (400). This study was designed as a multiple infusion to avoid the disequilibrium phase. Although the infusion rate was frequently changed in all of the test animals and there is a small decline in disopyramide binding with time in the human glycoprotein-treated rabbit, apparent steady state was observed in all rabbits at each stage allowing quantitative determination of the influence of altered serum binding on pharmacological

response.

In addition to supporting the use of unbound drug concentration in studying the concentration-response relationship, these data in general indicate that drug bound to serum proteins does not add to the magnitude of the pharmacological response. The use of total drug concentration in serum to predict the pharmacological response can be misleading if the serum protein binding is variable.

Chapter V.

Influence of Intrasubject Variation in Serum Protein Binding on the Pharmacological Response of R- and S-Disopyramide in the Rabbit

In the previous chapter, it was shown that pretreatment with human glycoprotein fraction VI significantly altered the relationship between the total disopyramide concentration in serum and the pharmacological effect in rabbits, but not the relationship between the unbound disopyramide concentration and the pharmacological response. The previous study indicates that the pharmacological response follows the change in the unbound drug concentration; and the total drug concentration-response will depend on the *interindividual difference* in serum protein binding. Based on the same notion, it can be postulated that a different pharmacological response before and after an alteration in serum protein binding will result in a subject only when there is an alteration in the unbound drug concentration. An intrasubject variation in the total serum drug concentration on the other hand is not always associated with a change in the pharmacological response. In this chapter, the serum protein binding of disopyramide was altered in individual rabbits and the pharmacological response before and after the binding alteration was compared. The studies were designed to reveal the influence of *intrasubject variation* in the serum protein binding on the pharmacological response.

It was shown in Chapter III that S-disopyramide is a high extraction ratio drug at low concentration and R-disopyramide is an intermediate extraction ratio drug. On treating rabbits with human glycoprotein fraction VI, the total serum concentration of R-disopyramide should increase and unbound serum concentration remain relatively constant, while the total serum concentration

of S-disopyramide should increase slightly and the unbound serum concentration should decrease. Both enantiomers are used as separate model drugs in this study. As discussed in Chapter III, a low concentration of S-disopyramide must be used to maximize the relative change in the unbound drug concentration, but the concentration of S-disopyramide has to be high enough to be able to quantitate the ΔQRS duration change. The dosing rate of S-disopyramide was therefore adjusted to maintain a serum concentration between 3 to 4 mg/ℓ in all rabbits studied. A dose of 80 mg/kg human glycoprotein fraction VI was administered in the studies of S-disopyramide to exaggerate the binding changes. The dosing rate of R-disopyramide, on the other hand, was adjusted to study the influence of altered serum protein binding on pharmacological response at various drug concentrations. A dose of 40 mg/kg human glycoprotein fraction VI was administered to each rabbit infused with R-disopyramide.

EXPERIMENTAL

Eleven New Zealand white rabbits weighing 2.35 to 3.2 kg were randomly divided into two groups. Six of them were infused with R-disopyramide into a marginal ear vein using a precalibrated pump (Harvard Model 975). To maintain various steady-state concentrations of R-disopyramide, different rates of R-disopyramide infusion, ranging from 0.095 to 0.32 mg/min/kg, were given for 185 minutes. A loading infusion, at approximately 3.8 times the maintenance infusion rate, was given for 15 minutes before maintenance infusion to achieve the desired steady-state concentration quickly. During the steady-state infusion (100 minutes after starting the infusion), 40 mg/kg human glycoprotein fraction VI in 3 ml water for injection was injected over 1 minute. Blood samples were taken prior to and 4, 8, 14, 20, 30, 50, 70, 90, 98, 102, 105, 120, 140, 160, and 180 minutes after the start of drug administration. Serum was then separated by centrifugation at 900 x g for 10 minutes. At 30, 50, 70, 90, 120, 140, 160, and 180 minutes, 3 ml blood were sampled for both disopyramide concentration determination and equilibrium dialysis; 1 ml blood was taken to determine disopyramide concentration in other samples.

Another group (5 rabbits) was given S-disopyramide infusion to achieve a steady-state concentration of 3 to 4 mg/ℓ (maintenance infusion rate 0.79 mg/min). A bolus dose of 80 mg/kg human glycoprotein fraction VI was administered at 100 minutes. The rest of the study protocol for S-disopyramide was identical to that for

R-disopyramide. One additional rabbit was given 80 mg/kg human glycoprotein fraction VI without disopyramide administration. Electrocardiographic response was measured in all rabbits throughout the study as described in Section II-4.

RESULTS

Injection of human glycoprotein fraction VI in rabbits decreased the unbound fraction of R-disopyramide; as a result, the steady-state concentration of total R-disopyramide in serum increased (Fig. V-1). The magnitude of increase ranged from 48% to 127%, depending on the initial R-disopyramide concentration. The unbound R-disopyramide concentration in serum showed little to no change (average 8.4%). The ΔQRS duration showed similarly an insignificant increase (8.4%). The data are listed in Table V-1 and the time profile of a representative rabbit is shown in Fig. V-1.

When 80 mg/kg human glycoprotein fraction VI was injected during S-disopyramide infusion, total S-disopyramide concentration in serum increased; while the unbound drug concentration and ΔQRS duration decreased in most of the rabbits (Table V-2 and Fig. V-2). The change of ΔQRS duration always followed the change in the unbound drug concentration.

The injection of 80 mg/kg human glycoprotein fraction VI did not change the QRS duration in the rabbit without disopyramide administration.

Table V-1. STEADY-STATE CONCENTRATION OF TOTAL R-DISOPYRAMIDE, UNBOUND R-DISOPYRAMIDE, AND Δ QRS DURATION CHANGES IN RABBITS BEFORE AND AFTER HUMAN GLYCOPROTEIN FRACTION VI TREATMENT

Rabbit	$C_{p,ss}$ (μg/ml)			$C_{u,ss}$ (μg/ml)			Δ QRS Duration (%)		
(#)	Before	After	% Change	Before	After	% Change	Before	After	% Change
110981	3.1	5.2	68.0	2.4	2.4	-0-	10.2	11.4	12.0
121981	3.7	8.4	127.0	3.1	3.8	23.0	16.3	16.4	0.6
112581	6.7	10.9	63.0	5.6	6.1	8.9	30.9	32.2	4.2
011782	6.9	11.3	64.0	5.8	6.0	3.4	30.7	35.3	15.0
011182	8.1	12.7	57.0	6.7	7.4	10.0	32.8	33.3	1.5
120281	9.3	13.8	48.0	7.9	8.3	5.1	48.7	57.0	17.0
MEAN			+76.0			+8.4			+8.4

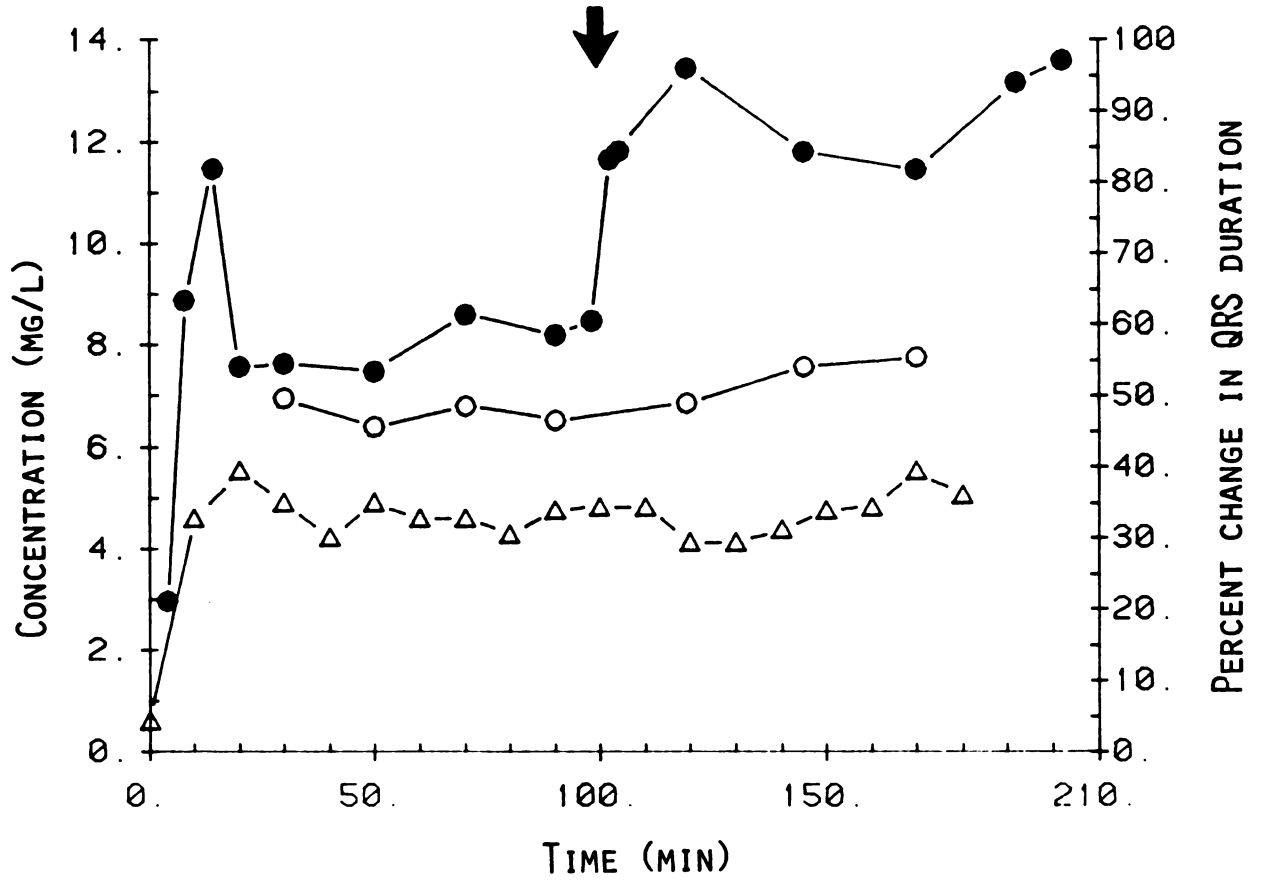


Fig. V-1. Injection of human glycoprotein fraction VI, 40 mg/kg (↓), increased the total concentration (●) of R-disopyramide, but not unbound concentration (○), nor ΔQRS duration (Δ).

Table V-2. STEADY-STATE CONCENTRATION OF TOTAL S-DISOPYRAMIDE, UNBOUND S-DISOPYRAMIDE, AND Δ QRS DURATION CHANGES IN RABBITS BEFORE AND AFTER HUMAN GLYCOPROTEIN FRACTION VI TREATMENT

Rabbit	$C_{p,ss}$ (mg/ℓ)			$C_{u,ss}$ (mg/ℓ)			Δ QRS Duration (%)		
(#)	Before	After	% Change	Before	After	% Change	Before	After	% Change
022682	3.8	7.6	100.	3.5	2.3	-34.	19.6	13.6	-30.
030282	4.1	7.9	93.	3.4	2.5	-26.	16.8	13.9	-17.
031882	4.1	9.8	139.	3.4	3.0	-12.	14.7	13.2	-10.
032582	5.5	7.2	31.	4.6	2.1	-54.	15.5	11.0	-29.
042682	4.2	8.8	110.	3.4	3.7	8.8	15.6	15.9	1.9
MEAN			95.			-23.			-17.

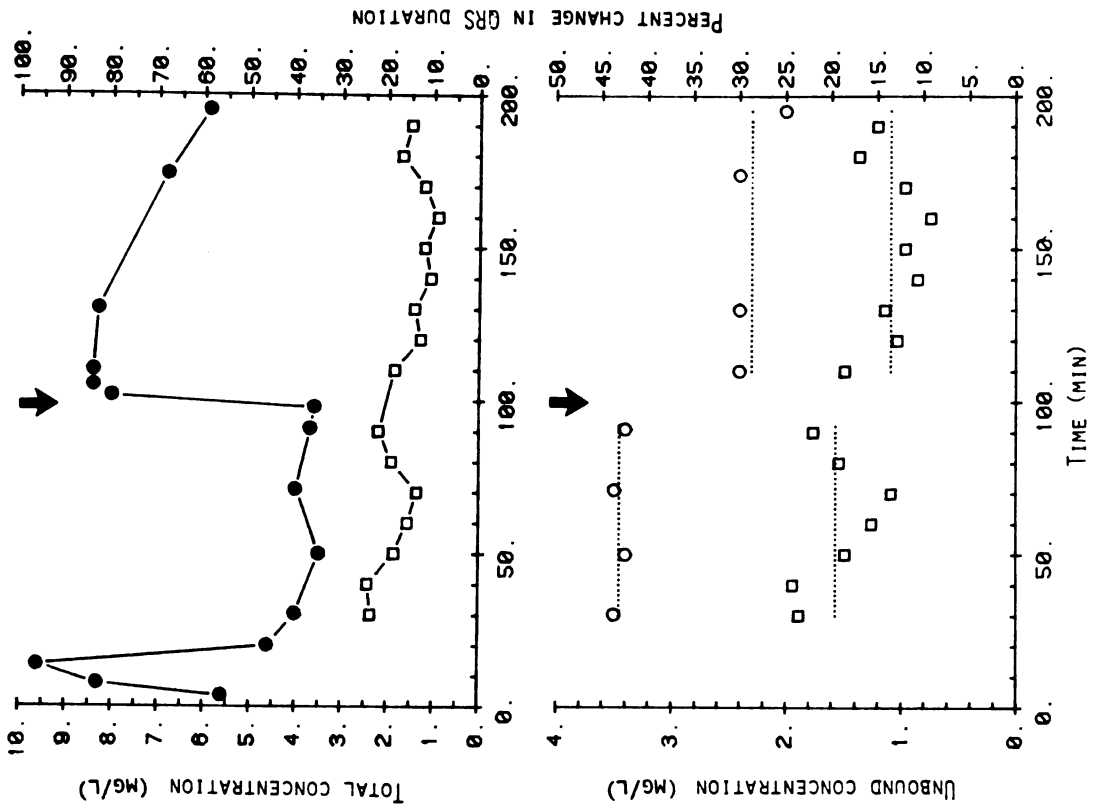


Fig. V-2. The injection of human glycoprotein fraction VI, 80 mg/kg (↓), increased the total concentration of S-disopyramide (● - upper panel); whereas the unbound drug concentration (o - lower panel) and ΔQRS duration (□ - both panels) decreased significantly.

DISCUSSION

This study indicates that when serum protein binding of disopyramide is altered, the pharmacological response changes in parallel with the changes of the unbound drug concentration. ΔQRS duration decreased when the unbound drug concentration of S-disopyramide decreased as a result of the binding changes. It slightly increased or remained constant when the unbound concentration of R-disopyramide or S-disopyramide remained constant or increased. Total drug concentration was dramatically increased under all conditions, again, indicating that the total drug concentration does not represent the pharmacological response during the variable serum protein binding condition and bound drug has no pharmacological activity.

The injection of 40 mg/kg human glycoprotein fraction VI does not alter the QRS duration in rabbits, which was shown in Fig. IV-2 and discussed in Chapter IV. The injection of 80 mg/kg human glycoprotein fraction VI to one additional rabbit confirms that human glycoprotein fraction VI itself has an effect on QRS duration only through altering the binding of disopyramide.

In the studies with R-disopyramide infusion, the injection of human glycoprotein fraction VI increased the total serum drug concentration. The magnitude of increase, ranging from 48% to 127%, however, depended on the concentration of R-disopyramide. That is due to the concentration-dependent binding of R-disopyramide

(Fig. III-3). Except for rabbit 110981 (Table V-1), the increase in total serum drug concentration is approximately the same for all other rabbits, reflecting the same concentration of drug bound to administered α_1-acid glycoprotein. The relative increase is higher when the drug concentration before glycoprotein treatment is low. The unexpected small increase in total serum drug concentration in rabbit 110981 can be explained if the extraction ratio of R-disopyramide is high for the rabbit; it is not due to the unusually low binding in the rabbit because the unbound fraction after treatment in rabbit 110981 is similar to the unbound fraction expected for the R-disopyramide concentration.

In studies with S-disopyramide infusion, the injection of human glycoprotein fraction VI generally decreased the unbound drug concentration and the pharmacological response. Rabbit 042682 is, however, an exception. It is possible that the extraction ratio of S-disopyramide in rabbit 042682 is substantially lower than in other rabbits, which would explain why the unbound drug concentration increased slightly instead of being decreased as in other rabbits. It is interesting to note that the pharmacological response follows the change of the unbound drug concentration whether it decreases or increases.

Apparent steady state was achieved for both R- and S-disopyramide concentrations before the alteration of serum protein binding; after human glycoprotein injection, another apparent steady state was quickly reached in each individual rabbit. Because R- and S-

disopyramide have exactly the same physico-chemical properties and binding to human glycoprotein preparation, the redistribution of R- and S-disopyramide in rabbits after human glycoprotein injection was assumed to be similar.

It has been generally hypothesized that when steady state is reached, unbound drug concentration at the site of drug action is equivalent to unbound drug concentration in serum. It is also generally assumed that unbound drug concentration at the site of drug action determines the magnitude of pharmacological response. The observations in this study and the study shown in the previous chapter are consistent with these concepts.

This study also suggests that it is the unbound drug concentration, not the total drug concentration, in serum that should be used for therapeutic monitoring or evaluation in man for drugs with variable serum protein binding. The use of total serum drug concentration to determine a dosage regimen could be misleading and dangerous for patients on drugs with a low therapeutic range if the serum protein binding changes.

In conclusion, it is the unbound drug concentration that is relevant to measure in order to describe the pharmacological response during a steady-state condition. Whenever drug concentration is used to predict pharmacological effects, the serum protein binding of the drug should not be ignored.

Chapter VI.

Hepatic Elimination of Drugs with Concentration-Dependent Serum Protein Binding

Based on the concept of perfusion-limited drug elimination, two physiological models, the "well-stirred" model and "parallel tube" model, have been proposed to explain the empirical observations in hepatic elimination of drugs (Section I-1-b). The two corresponding equations, Eqs. I-8 and I-13, were also proposed to relate hepatic blood clearance with hepatic blood flow, unbound fraction of drug in blood, and the intrinsic clearance. These two equations, however, were derived under the assumption of linear drug binding, i.e., that the unbound drug fraction in blood entering the liver is identical to the unbound drug fraction inside the sinusoids. This condition is not met when the vascular concentration of drug is in a nonlinear range and the concentration difference entering and leaving the liver is sufficiently large to give rise to significant concentration-dependent binding differences.

As discussed in Section I-4-b, the unbound fraction increases as the drug concentration increases for a concentration-dependent binding drug. From Eqs. I-8 and I-13, it is apparent that as the concentration and the unbound fraction of drug in blood increase, the hepatic clearance and extraction ratio increase as well. The first pass availability therefore decreases. When significant changes in the unbound fraction of drug in blood are encountered, the unbound fraction inside the liver at any time is lower than the unbound fraction of drug in blood entering the liver, which is usually the unbound fraction value that is determined. The clearance and extraction ratio in the liver, under such conditions, is smaller than expected from Eqs. I-8

and I-13 at a given unbound fraction in blood. The hepatic first pass, on the other hand, is larger than expected.

In this chapter, the equations for both "well-stirred" model and "parallel tube" model are reformulated with the consideration of concentration-dependent serum protein binding. The maximal effect of concentration-dependent binding as well as the influence of variables affecting the hepatic elimination of drugs are discussed.

SIMULATIONS

In the discussion below, the following assumptions have been made:

1. The ratio of erythrocyte volume to whole blood volume, hematocrit, H, is a constant at all times and drug concentrations.
2. The drug linearly partitions into erythrocytes, i.e., the ratio of the drug concentration in erythrocytes to that unbound in serum water, λ, is independent of the drug concentration.
3. There is only one class of drug binding sites in serum. The total concentration of binding sites, Pt, and the dissociation constant of the drug-binding site complex, Kd, are the same in blood entering and leaving the liver, as well as in the sinusoids.
4. The binding of drug to the serum protein follows the law of mass action.

Well-stirred model. The basic assumption for the "well-stirred" model is that the unbound drug concentration in the sinusoid is identical to the concentration leaving the liver. Based upon this assumption, the well-stirred model can readily be shown to be described by:

$$Cl_{HB} = \frac{Q \cdot \alpha_{Bv} \cdot Cl_I}{Q + \alpha_{Bv} \cdot Cl_I} \qquad \text{(Eq. VI-1)}$$

where α_{Bv} is the unbound fraction of drug in blood in the sinusoid and in blood leaving the liver. As α_{Bv} cannot readily be measured, the unbound fraction of drug in blood entering the liver, α_B,

is usually assumed to be equal to α_{Bv}. When concentration-dependent binding is encountered, this is at best an approximation. Under conditions of large changes in the concentration as it passes through the liver, the unbound fraction α_{Bv} can be substantially different from α_B. To calculate the relationship between α_B and α_{Bv}, the following relationships are needed:

$$\alpha = \frac{Cu + Kd}{Cu + Kd + Pt} \qquad \text{(Eq. VI-2)}$$

$$\alpha_{Bv} = \frac{Cu_v + Kd}{Cu_v + Kd + Pt} \qquad \text{(Eq. VI-3)}$$

$$Cu = C_B \cdot \alpha_B \qquad \text{(Eq. VI-4)}$$

$$Cu_v = C_{Bv} \cdot \alpha_{Bv} \qquad \text{(Eq. VI-5)}$$

$$C_{Bv} = C_B \cdot (1 - \frac{Cl_{HB}}{Q}) \qquad \text{(Eq. VI-6)}$$

$$\alpha_B = \frac{\alpha}{1 - H + H \cdot \lambda \cdot \alpha} \qquad \text{(Eq. VI-7)}$$

$$\alpha_{Bv} = \frac{\alpha_v}{1 - H + H \cdot \lambda \cdot \alpha_v} \qquad \text{(Eq. VI-8)}$$

where Cu is the unbound concentration in blood, C_B is the blood concentration, and the subscript v denotes concentrations and unbound fractions leaving the liver. No subscript denotes concentration or unbound fraction entering the liver. α and α_v are the unbound fraction of drug in plasma, instead of blood.

The relationship between hepatic clearance and the unbound fraction of drug entering the liver was determined solving equations VI-1 through VI-8 by iterative processes.

To illustrate the influence of concentration-dependent binding on hepatic elimination of drugs, a situation in which the unbound drug concentration is much greater than the dissociation constant and λ is equal to 0 is considered. Under these conditions, the combination of Eqs. VI-1 to VI-8 can be solved explicitly and gives:

$$Cl_{HB} = \frac{Q \cdot \alpha \cdot Cl_I}{Q \cdot (1 - H) + Cl_I} \quad . \qquad \text{(Eq. VI-9)}$$

See Appendix for derivation.

Parallel tube model. In the "parallel tube" model the ability to metabolize drug is assumed to be identical along the length, L, of the sinusoids in the liver. The rate of elimination can then be expressed as:

$$Q\, dC_{Bx} = - Cu_x \cdot \left(\frac{Cl_I}{L}\right) dx \qquad \text{(Eq. VI-10)}$$

where the subscript x denotes concentrations and unbound fractions of drug at a distance x from the entrance of the sinusoid.

By using the following additional relationships:

$$\alpha_{Bx} = \frac{Cu_x}{C_{Bx}} = \frac{\alpha_x}{1 - H + H \cdot \lambda \cdot \alpha_x} \qquad \text{(Eq. VI-11)}$$

$$\alpha_x = \frac{Cu_x + Kd}{Cu_x + Kd + Pt} \qquad \text{(Eq. VI-12)}$$

and substituting Eqs. VI-11 and VI-12 into Eq. VI-10, and intergrating from 0 to L, one obtains:

$$\frac{Cl_I}{Q} = (1 - H + H\cdot\lambda)\ ln\ \frac{Cu}{Cu_v} + (1-H)\cdot\frac{Pt}{Kd}\ ln\ \frac{Cu\cdot(Kd + Cu_v)}{Cu_v\cdot(Kd + Cu)}$$

$$+ (1 - H)\cdot Pt\cdot\left(\frac{1}{Kd + Cu} - \frac{1}{Kd + Cu_v}\right) \quad . \qquad \text{(Eq. VI-13)}$$

Utilizing Eqs VI-2 to VI-8, together with Eq. VI-13, the relationship between hepatic clearance of drugs and the unbound fraction of drug entering the liver was solved by iterative methods.

For illustrative purposes, the situation in which the unbound drug concentration is much greater than the dissociation constant and λ is equal to 0 is presented. Under these conditions, the hepatic blood clearance can be expressed by:

$$Cl_{HB} = Q\cdot\alpha\cdot\left(1 - exp\ \left(-\frac{Cl_I}{Q\cdot(1-H)}\right)\right) \quad . \qquad \text{(Eq. VI-14)}$$

See Appendix for derivation.

Simulations. The following situations were simulated using a PDP-11 computer and the PROPHET system:

1. Determination of the extraction ratio as a function of the unbound fraction at various intrinsic clearance values. The relationships are carried out at two extremes: (a) when the unbound fraction is essentially independent of changes in the drug concentration; and (b) when the binding protein is mostly saturated, *i.e.*, when Eqs. VI-9 and VI-14 are valid.
2. Determination of the extraction ratio as a function of the unbound fraction at various Cu/Kd values.

3. Determination of the extraction ratio as a function of drug concentration for a given concentration of binding sites.
4. Determination of the extraction as a function of the unbound fraction when λ is equal to 0 and when λ is equal to 1.

In all simulations, a hematocrit of 0.45 was assumed, and the dissociation constant of the binding was set at 1 μM. The values of other parameters were specified in each simulation.

RESULTS AND DISCUSSION

The relationship between extraction ratio and unbound fraction for drugs with linear or concentration-dependent serum protein binding are shown in Fig. VI-1 using the "well-stirred" model and "parallel tube" model at various intrinsic clearance values. It is apparent that the larger the intrinsic clearance values, the more important it is to recognize concentration-dependent binding during the hepatic elimination process. As the intrinsic clearance tends toward infinity, a drug with linear protein binding approaches an extraction ratio of 1 at all values of the unbound fraction, whereas a drug with concentration-dependent binding and when the unbound concentration of the drug is much greater than the dissociation constant will have an extraction ratio equal to the unbound fraction of drug in plasma. The discrepancy in the the extraction ratio can therefore be as much as 1 between two drugs with the same intrinsic clearance, unbound fraction, and unbound drug concentration but being different in the dissociation equlibrium constant of the binding complexes.

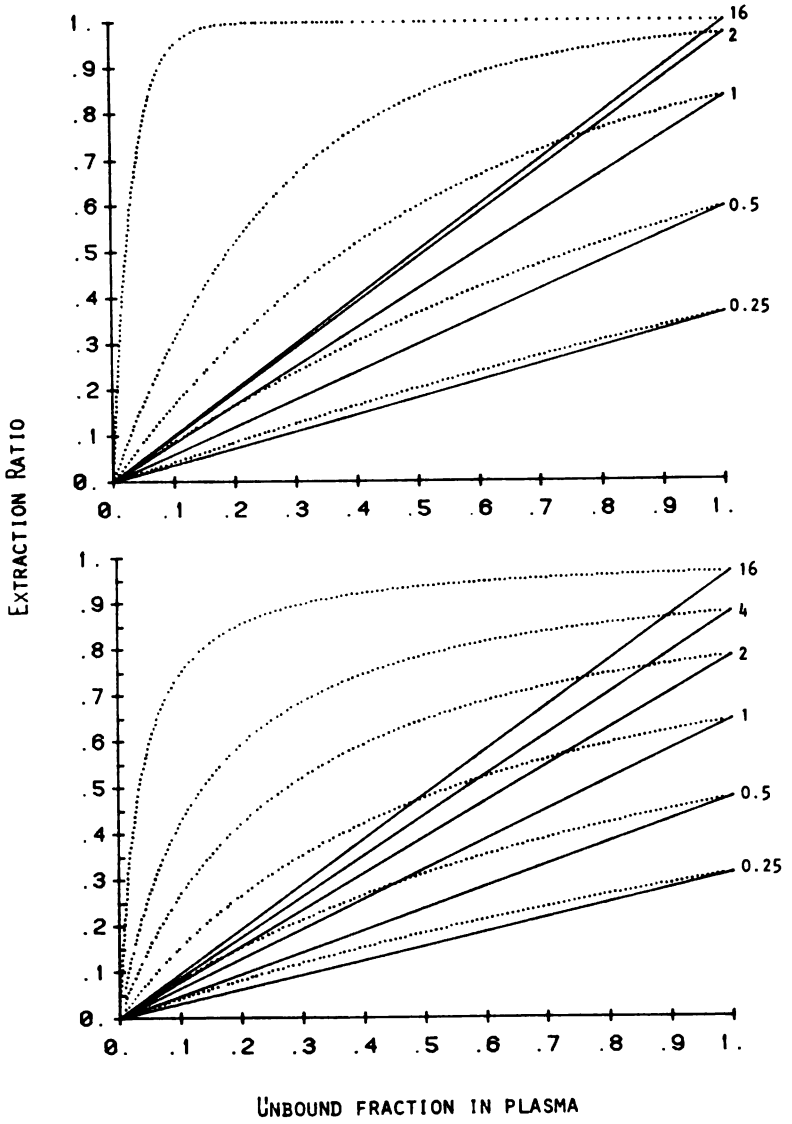
Both Eqs. VI-9 and VI-14 can be simplified to:

$$Cl_{HB} = \alpha \cdot Q \qquad \text{(Eq. VI-15)}$$

$$Cl_{u} = Q \cdot (1-H) \qquad \text{(Eq. VI-16)}$$

when intrinsic clearance is much greater than blood flow. The hepatic clearance, as well as the extraction ratio, under these conditions is directly proportional to the unbound fraction in

Fig. VI-1. Extraction ratio of drugs with linear protein binding (---) compared to that of drugs with concentration-dependent binding and when C_u is much greater than K_d and λ approaches 0 (——). *Upper panel.* Predictions based on the "parallel tube" model. *Lower panel.* Predictions based on the "well-stirred" model. Numbers at the right of curves are intrinsic clearance values in multiples of hepatic blood flow.



plasma, whether the drug has high or low extraction ratio characteristics; and the unbound clearance depends on blood flow. On the other hand, a drug with concentration-dependent binding which has a low extraction ratio at low unbound fraction range may actually have a high intrinsic clearance. The hepatic clearance will then be flow-dependent as Eq. VI-15 shows and the unbound clearance will be determined by blood flow instead of intrinsic clearance as Eq. I-11 predicts for a linear-binding drug.

The discussion above has focused on the highest influence of concentration-dependent binding, i.e., when the unbound drug concentration is much greater than the dissociation constant and there is no drug partitioning into red blood cells. Practically, the extraction ratio of a concentration-dependent binding drug will be between the two extremes of prediction which are shown in Fig. VI-1. In Fig. VI-2, the extraction ratio as a function of unbound fraction was calculated at various Cu/Kd values. It appears that the higher the drug concentration, or the lower the dissociation constant, the more important it is to consider the concentration-dependent binding during the hepatic elimination process.

In Fig. VI-3, the dependence of the hepatic extraction ratio on the drug concentration for a concentration-dependent binding drug was simulated, assuming the intrinsic clearance is not concentration-dependent. The data demonstrate that consideration of concentration-dependent serum protein binding during the

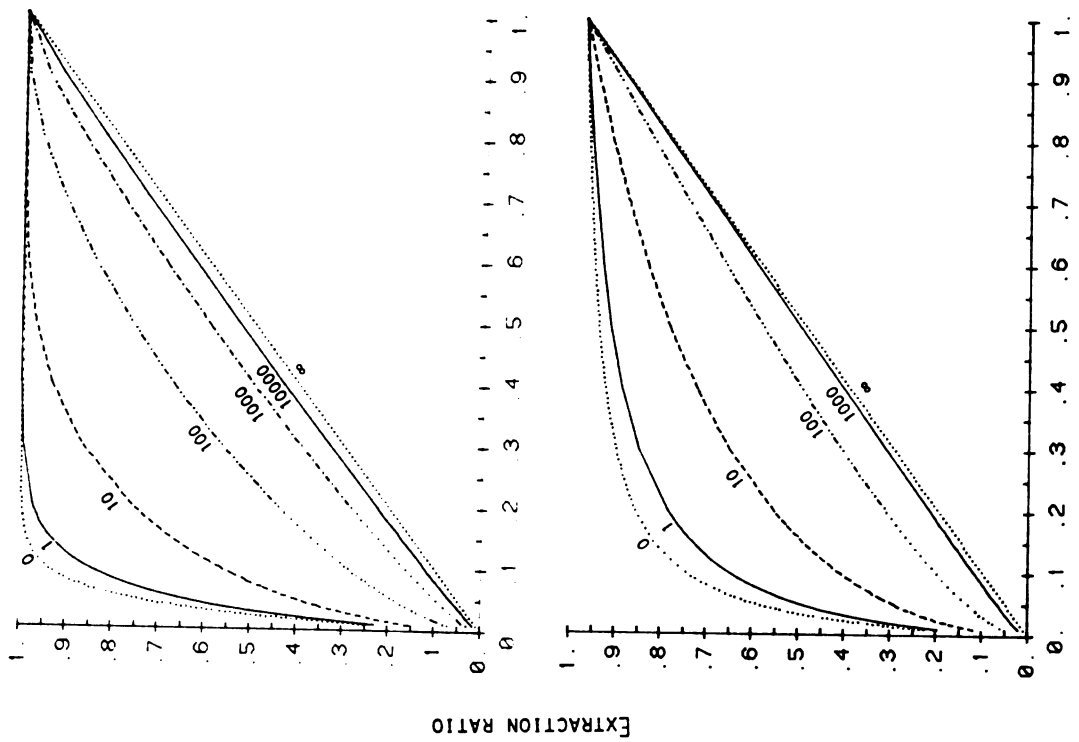


Fig. VI-2. Relationship between the hepatic extraction ratio and the unbound fraction at various ratios of drug concentration to the dissociation constant. Number adjacent to each curve is the ratio. Intrinsic clearance is 16 times the blood flow; $\lambda = 0$. *Upper panel*. Relationships predicted by the "parallel tube" model. *Lower panel*. Relationships predicted by the "well-stirred" model.

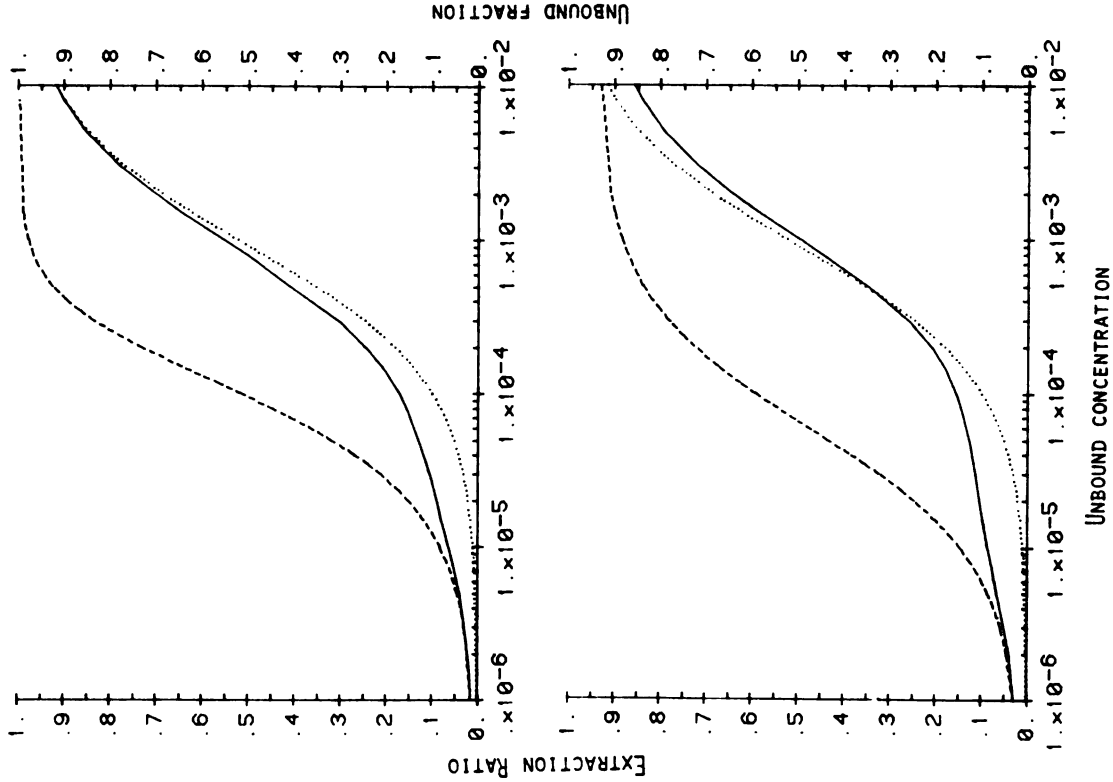


Fig. VI-3. Influence of drug concentration on the hepatic extraction ratio of a concentration-dependent binding drug. Concentration of binding sites is 909 μM. (·····): Unbound fraction at various concentrations. Solid and broken lines: Predicted extraction ratio-concentration relationships when elimination depends upon the unbound fraction entering the liver (---) or depends upon the unbound fraction inside the sinusoid (——). *Upper panel*. Simulation based on the "parallel tube" model; intrinsic clearance is 4 times the blood flow. *Lower panel*. Simulations based on the "well-stirred" model; intrinsic clearance is 8 times the blood flow.

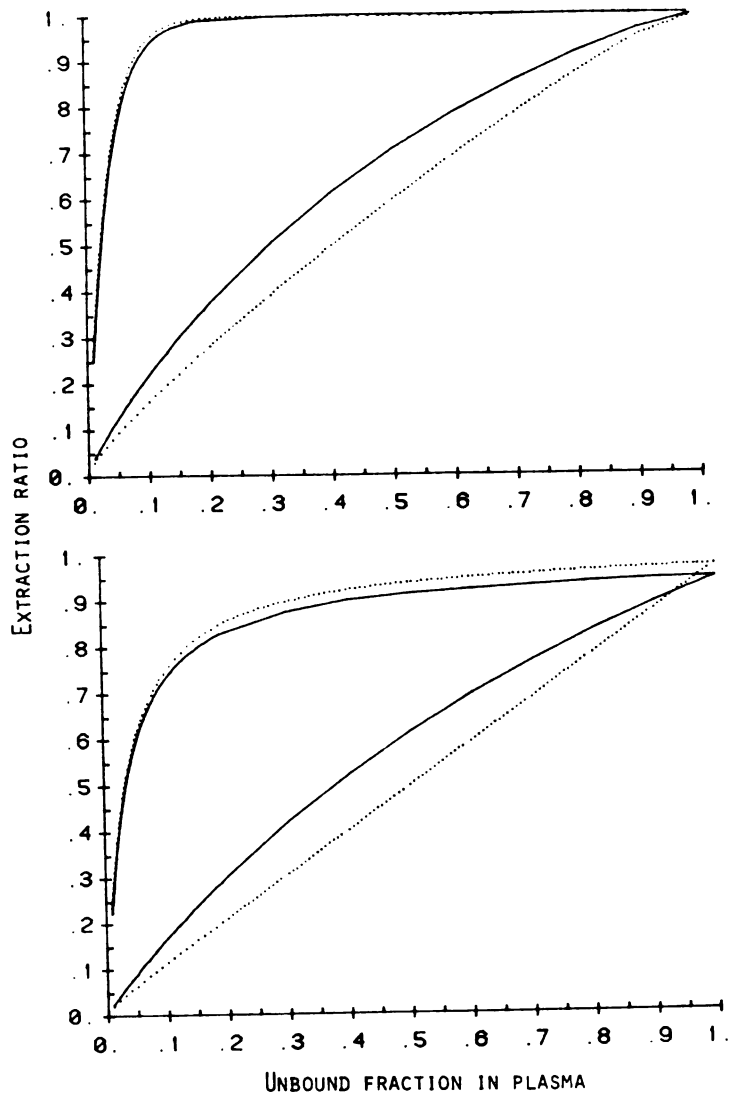
hepatic elimination process is important; predictions based on Eqs. I-8 and -13 (without considering the binding changes during the elimination process) give a much higher value for the extraction ratio than predictions based on equations presented in this chapter.

Concentration-dependent binding also has consequences for how the plasma concentration declines with time for high intrinsic clearance drugs. McNamara *et al* (249) and Øie *et al* (278) simulated the plasma concentration-time profile for drugs with concentration-dependent binding without considering that the unbound fraction inside the sinusoids is lower than the unbound fraction in blood entering the liver. Because the clearance is much smaller in the "intermediate" concentration range than was predicted previously (Fig. VI-3), the decline of plasma concentrations for high intrinsic clearance drugs are, therefore, overdramatized at certain concentration ranges. The actual decline for this type of drug is less than that suggested by above authors, although the general shape of the curves is not different.

Simulations in Figs. VI-1 to -3 have been restricted to situations where drug is not partitioned into red blood cells to emphasize the importance of concentration-dependent binding. As Eqs. VI-2 and -7 show, the higher the value of λ, the less concentration-dependent is the unbound fraction in blood. The influence of concentration-dependent serum protein binding will be less important. However, it is important to realize that assumption 2 in this chapter, that partitioning into red blood cell is linear, is not

necessarily true, as the partitioning of drug into red blood cell may well be concentration-dependent. When concentration-dependent binding to red blood cells is encountered, the effect of such nonlinear binding will give rise to values similar to those simulated for nonlinear plasma protein binding. To illustrate that the zero partitioning of drug into red blood cells is not required for concentration-dependent serum protein binding to affect hepatic elimination significantly, the situations in which the partition coefficient is equal to 0 or 1 are simulated and compared in Fig. VI-4. The results show that the linear partitioning of drug into the red blood cells decreases the influence of concentration-dependent serum protein binding, but a significant effect is still seen.

Fig. VI-4. Influence of concentration-dependent binding on the hepatic extraction ratio of drugs with different extents of red blood cell partitioning: (——): $\lambda = 1$; (···): $\lambda = 0$. Intrinsic clearance is 16 times blood flow for all curves. In each panel, the upper pair of curves is the relationship for drugs with linear binding and the lower pair drugs with concentration-dependent binding under conditions of $C_u / K_d = 1000$. *Upper panel.* Simulations based on "parallel tube" model. *Lower panel.* Simulations based on "well-stirred" model.



Chapter VII.

Influence of Serum Protein Binding on Hepatic Clearance of S-Disopyramide in the Rabbit

The influence of serum protein binding on hepatic clearance of drugs has been proposed using flow models (Section I-1-b). Based on the same concepts, the influence of serum protein binding on the hepatic clearance of drugs with concentration-dependent binding was proposed in Chapter VI.

Most *in vivo* studies undertaken to determine the relationship between the hepatic clearance of drugs and the unbound fraction in plasma have been consistent with the proposed models (144,222, 228,279,417). These correlations can unfortunately only be considered as "casual" evidence of the validity of the models; as the range of unbound fraction studied are usually achieved by selecting subjects with a wide variation in the unbound fraction in plasma, the conclusions drawn are heavily dependent upon there being little interindividual variation in the intrinsic clearance or hepatic blood flow. Experiments in this chapter were carried out to study a cause-effect relationship of altered serum protein binding to the drug clearance in the same animals. S-Disopyramide was chosen as the test drug and rabbits as the test animal in these studies.

EXPERIMENTAL

Animal experiments. Four New Zealand white rabbits weighing 2.55 to 3.25 kg were used in this study. S-Disopyramide solution was infused into a marginal ear vein using a precalibrated pump (Harvard Model 975). To maintain various steady-state concentrations of S-disopyramide, different rates of S-disopyramide infusion, ranging from 0.027 to 0.099 mg/min/kg, were used. A loading infusion, approximately 3.8 times the maintenance infusion rate, was given for 15 minutes to achieve the desired steady-state concentration quickly. During the steady-state infusion, doses of 13, 27, 40 mg/kg human glycoprotein fraction VI in sterile water were injected at 80, 160, and 240 minutes, respectively. Blood samples were taken prior to and 40, 50, 60, 70, 120, 130, 140, 150, 200, 210, 220, 230, 280, 290, 300, and 310 minutes after the start of drug administration. At 40, 60, 120, 140, 200, 220, 280, and 300 minutes, 1 ml blood was sampled and frozen immediately for determination of disopyramide concentration in the whole blood. At all other times, three ml blood was taken. One ml was frozen for blood disopyramide concentration assay and the remainder was centrifuged and the serum removed for determination of S-disopyramide total and unbound concentration. Hematocrit was measured for each blood sample. Urine was collected for the last 50 minutes at each steady-state level and the amount of S-disopyramide excreted unchanged determined.

Measurement of indocyanine green clearance. Indocyanine green clearance was determined during each steady-state infusion period

using the following method. A 0.1 mg/kg dose of indocyanine green (ICG) was dissolved in 0.3 ml sterile water and injected into a marginal ear vein in the rabbit within 3 seconds. S-Disopyramide infusion was temporarily stopped during the ICG injection. Blood was collected from the marginal ear vein in the other ear by continuous withdrawl using a Harvard infusion/withdrawal pump (Model 931) at a speed of 0.36 ml/min over an 8-minute period. The plasma was separated from blood by centrifugation at 300 x g for 10 minutes. The hematocrit (H) was determined and ICG concentration was assayed as described in Section II-5. The blood clearance of ICG ($Cl_{B,ICG}$) was then calculated using the following equation:

$$Cl_{B,ICG} = \frac{\text{dose}}{\bar{C}_{p,ICG} \cdot \tau \cdot (1-H)} \qquad \text{(Eq. VII-1)}$$

where τ is the withdrawl time and $\bar{C}_{p,ICG}$ is the plasma concentration in the sample (averaged plasma concentration over the total collection period).

Data analysis. The total body clearance (Cl_{TB}) of S-disopyramide was calculated by the steady-state blood concentration ($C_{B,ss}$) using:

$$Cl_{TB} = \frac{R}{C_{B,ss}} \qquad \text{(Eq. VII-2)}$$

where R is the S-disopyramide dosing rate. The fraction of S-disopyramide excreted in urine unchanged (fe) is determined by the amount of S-disopyramide recovered in the urine sample divided by the amount of S-disopyramide administered during each steady-state period. The nonrenal clearance, which is assumed to be hepatic clearance

(Cl_{HR}), was then calculated using:

$$Cl_{HB} = Cl_{TB} \cdot (1 - fe) \quad . \quad \text{(Eq. VII-3)}$$

The unbound fraction of S-disopyramide (α), measured by equilibrium dialysis, was corrected for volume shift and concentration-dependence using a method similar to that described by Tozer *et al* (424). Assuming there is only one class of binding sites and the binding follows the law of mass action, the concentration of binding sites (Pt) inside the dialysis cell at equilibrium was calculated using an equation rearranged from Eq. I-22:

$$Pt = (Kd + Cu) \cdot \left(\frac{1}{\alpha} - 1\right) \quad \text{(Eq. VII-4)}$$

where Cu is the S-disopyramide concentration measured in the buffer side after equilibrium dialysis, and Kd is the dissociation constant of S-disopyramide-α_1-acid glycoprotein complex in rabbit serum. The value of Kd was determined in Chapter III to be 4.7 μM. Because there is consistently a 10% water flux from the buffer side into the serum side in the equilibrium dialysis cells, the corrected binding-site concentration (Pt*) is:

$$Pt^* = Pt \times 1.1 \quad . \quad \text{(Eq. VII-5)}$$

The unbound drug concentration in serum (Cu*) corrected for volume shift and concentration-dependence was then calculated by another rearrangement of Eq. I-22:

$$Cu^* = \frac{Cp - Kd - Pt^* + \sqrt{(Cp - Kd - Pt^*)^2 + 4 \cdot Cp \cdot Kd}}{2} \quad \text{(Eq. VII-6)}$$

where Cp is the total S-disopyramide concentration measured in serum

before equilibrium dialysis. The corrected unbound fraction of S-disopyramide in serum (α^*) was calculated by:

$$\alpha^* = \frac{Cu^*}{Cp} \quad . \qquad \text{(Eq. VII-7)}$$

The unbound fraction of S-disopyramide in blood (α_B^*) was obtained from:

$$\alpha_B^* = \frac{Cu^*}{C_B} \quad . \qquad \text{(Eq. VII-8)}$$

The observed hepatic blood clearance was compared with the theoretically-predicted values using the "well-stirred" model and "parallel tube" model. The hepatic blood flow was obtained from the indocyanine green clearance assuming an extraction ratio of indocyanine green of 0.9 (24,144). The intrinsic clearance of S-disopyramide was calculated using Eqs. I-8 and I-13 from steady-state S-disopyramide levels prior to glycoprotein injection; at this time, the serum protein binding of S-disopyramide is linear. The intrinsic clearance was assumed to be constant throughout the study in each rabbit. Equations I-8, I-13, VI-1 to VI-8, and VI-13 were used to calculate the predicted hepatic blood clearance with and without considering concentration-dependent binding in the elimination process based on the "well-stirred" model and "parallel tube" model.

RESULTS

The corrected unbound fractions, unbound drug concentration, the observed hepatic blood clearance, and the averaged indocyanine green clearance are listed in Table VII-1. The relationship between the hepatic clearance and the unbound fraction of S-disopyramide in blood is plotted in Fig. VII-1 and compared with theoretically-predicted values based on the "well-stirred" and "parallel tube" models.

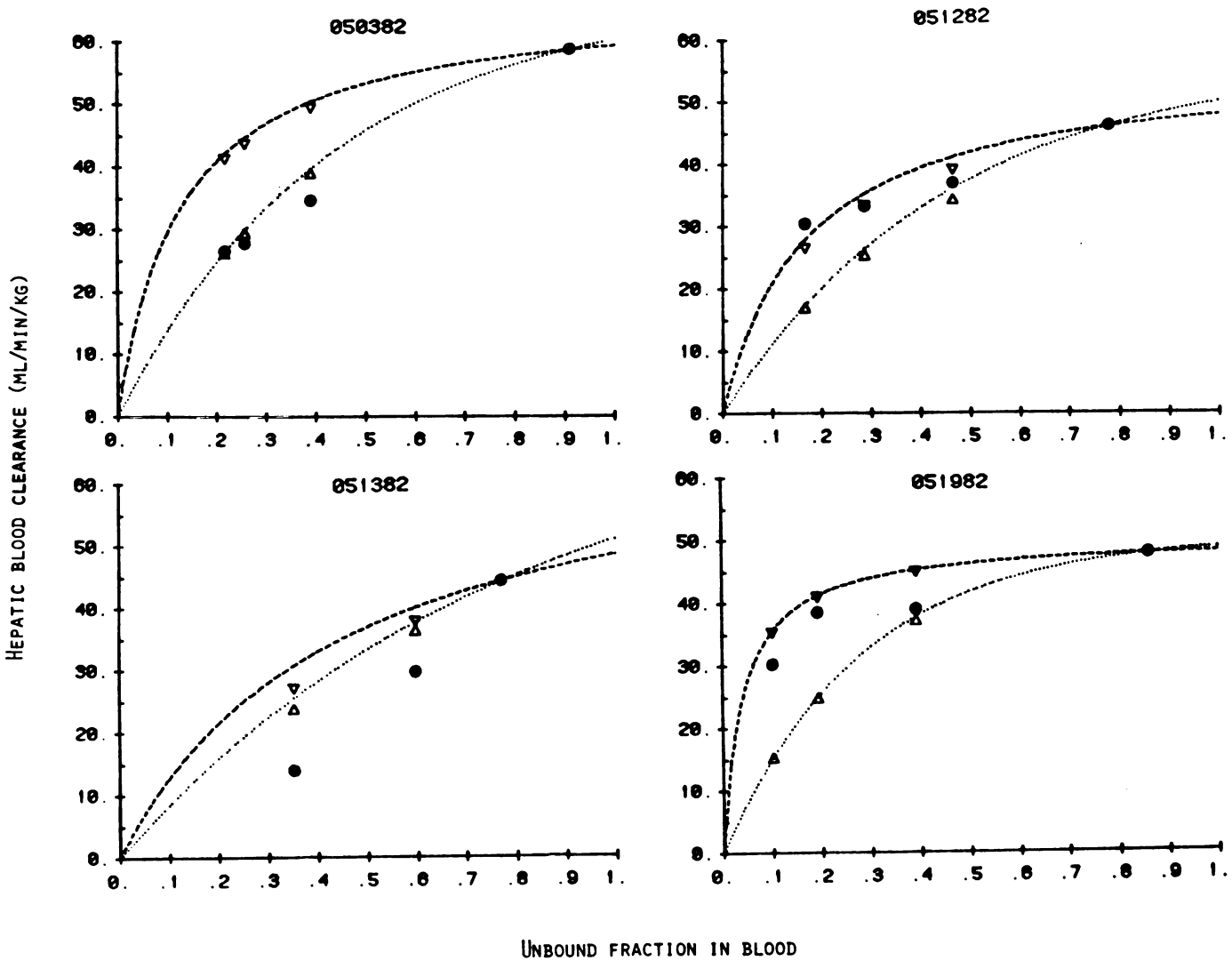
It is apparent that the hepatic blood clearance decreased as the unbound disopyramide fraction was decreased by the injection of human glycoprotein fraction VI. In general, the observed relationship is consistent with the proposed models; no distinction between the "well-stirred" and "parallel tube" models can be concluded, neither can predictions with and without consideration of concentration-dependent binding during the elimination process be distinguished.

Table VII-1. OBSERVED PHARMACOKINETIC PARAMETERS OF S-DISOPYRAMIDE AND CLEARANCE OF INDOCYANINE GREEN IN RABBITS TREATED WITH HUMAN GLYCOPROTEIN FRACTION VI

Rabbit (#)	Study Period (min)	α*	α_B*	C_u* (mg/ℓ)	Cl_{HB} (ml/min/kg)	$Cl_{B,ICG}$† (ml/min/kg)
050382	0-80	0.91	0.91	0.43	59	60 ± 7.2
	80-160	0.29	0.39	0.32	35	
	160-240	0.18	0.26	0.23	28	
	240-320	0.15	0.22	0.22	26	
051282	0-80	0.79	0.78	1.4	46	50 ± 8.8
	80-160	0.40	0.47	1.1	37	
	160-240	0.22	0.29	0.64	33	
	240-320	0.12	0.17	0.45	30	
051382	0-80	0.80	0.77	1.6	44	63 ± 10
	80-160	0.53	0.60	1.5	30	
	240-320	0.26	0.35	2.0	14	
051982	0-80	0.80	0.86	0.43	48	45 ± 3.9
	80-160	0.29	0.39	0.25	39	
	160-240	0.13	0.19	0.14	39	
	240-320	0.066	0.10	0.092	30	

†Mean ± S.D. for each rabbit studied.

Fig. VII-1. Relationship between hepatic blood clearance and protein binding in blood in four rabbits treated with human glycoprotein fraction VI. (●): Observed data. (Δ): Values predicted by the "parallel tube" model under concentration-dependent protein binding conditions (Eq. VI-13). (·····): predicted values for the "parallel tube" model with linear binding during the extraction process. (▽): Values predicted by the "well-stirred" model under concentration-dependent protein binding conditions (Eqs. VI-1 to -8). (---): Predicted values for the "well-stirred" model with linear binding during the extraction process.



DISCUSSION

When serum protein binding of S-disopyramide in rabbits was altered by human glycoprotein treatment, hepatic blood clearance was also changed; the relationship between the clearance and the drug binding varied from rabbit to rabbit. The data suggest that serum protein binding is one of the physiological factors determining the hepatic elimination of drugs. However, there may be other variables that can affect the hepatic clearance of drugs and the drug binding-clearance relationship.

Indocyanine green clearance, which has been used to assess the hepatic blood flow in the rabbit (144), was measured in this study. As no significant changes in the indocyanine green clearance before and after glycoprotein treatment has been observed in any of the rabbits, no alteration in the hepatic blood flow was assumed. The decrease in the hepatic clearance of S-disopyramide is therefore not considered to be due to the change in the hepatic blood flow.

Because successive administrations of the same dose of human glycoprotein produced gradually decreasing changes in the unbound fraction, the dose of human glycoprotein was increased in steps. The last and largest dose of human glycoprotein often changed the hepatic clearance, as well as the unbound fraction, less than the first and smallest dose of human glycoprotein. Although intrinsic clearance may be altered following the human glycoprotein injection,

it is unlikely that the small dose of human glycoprotein changes the intrinsic clearance more than the large dose does. Therefore, it appears that the decrease in the hepatic clearance after the human glycoprotein injection is due to the unbound fraction changes.

Indocyanine green clearance is approximately 80 to 100% of hepatic blood flow in the rabbit (24,144). An average value, 90%, was used to estimate hepatic blood flow from the indocyanine green clearance in this study.

The hepatic blood clearance of S-disopyramide in rabbits after human glycoprotein treatment can be predicted by unbound fraction based on the "well-stirred" and "parallel tube" models assuming that the intrinsic clearance and blood flow are constant throughout the study period. As shown in Fig. VII-1, the change in the hepatic clearance of S-disopyramide is in general consistent with the theoretical predictions. Rabbits 050382 and 051382 appear to follow the prediction of the "parallel tube" model better, while rabbits 051282 and 051982 appear to follow the change predicted by the "well-stirred" model. The superiority of neither model can be concluded. In this study, the choice of the model depends on the estimated blood flow which cannot be independently verified. Inaccuracy in blood flow measurement explains, at least in part, the inconsistency in model selection in these rabbits.

S-Disopyramide shows a concentration-dependent serum protein

binding in rabbits after human glycoprotein treatment (Fig. III-3). As proposed in Chapter VI, predictions which do not consider concentration-dependent binding during the elimination process based on the "well-stirred" model (Eq. I-8) and the "parallel tube" model (Eq. I-13) give a higher value of clearance than predictions based on equations proposed in Chapter VI (Fig. VII-1). The discrepancy is, however, small in this study because of significant partitioning of S-disopyramide into blood cells ($\lambda>1$) and low Cu/Kd values. Only in rabbit 051382, which had a low hematocrit (0.33) and a slightly higher drug concentration, was a small discrepancy between predictions seen. The low drug concentration used in this study ensured that the unbound fraction of S-disopyramide measured was close to the unbound fraction in sinusoids and avoided further complications in the relationship between the hepatic clearance and unbound fraction of drugs.

Summary

Disopyramide is a drug which shows little binding in the rabbit serum (unbound fraction 85%) but shows concentration-dependent serum protein binding in man. α_1-Acid glycoprotein is the main binding protein in human serum. Injection of human glycoprotein fraction VI, which contains α_1-acid glycoprotein, into rabbits increased the serum disopyramide binding and the binding became concentration-dependent. No difference in binding of the two enantiomers of disopyramide, R-disopyramide and S-disopyramide, to rabbit serum or to human glycoprotein was found (Fig. III-3, Tables III-1 and III-2).

Both R- and S-disopyramide prolong QRS duration of the rabbit electrocardiogram. R-Disopyramide is more potent than S-disopyramide at high concentration, but equally potent when the concentration is low (Fig. III-1). The relative change in QRS duration (ΔQRS duration) was used to quantitate the pharmacological response in the rabbit to disopyramide. The relative change in ΔQRS duration before and after the glycoprotein treatment was used to quantitate the influence of serum protein binding on the pharmacological response.

R-Disopyramide is an intermediate extraction ratio drug in the rabbit, the extraction ratio is relatively concentration-independent. S-Disopyramide is a high extraction ratio drug at low concentration (below 4 mg/ℓ) and intermediate extraction ratio drug at high drug concentration (Fig. III-2).

One group of rabbits were treated with 40 mg/kg human glycoprotein fraction VI to increase the serum proteon binding of disopyramide. A second group, not given human glycoprotein fraction VI, served as a control. The disopyramide total concentration-response relationship was found to be different in the two groups (Fig. IV-6), while the unbound concentration-response relationship of disopyramide was the same in both groups of rabbits (Fig. IV-5). The human α_1-acid glycoprotein in the rabbit serum increased the bound disopyramide concentration but not the unbound disopyramide concentration or ΔQRS duration. The experiment indicates that the bound drug concentration is unimportant for estimating the effect of disopyramide.

During the steady-state infusion of R-disopyramide in the rabbit, the injection of 40 mg/kg human glycoprotein fraction VI increased the total drug concentration in serum, but did not change the unbound drug concentration or ΔQRS duration (Fig. V-1). During the steady-state infusion of S-disopyramide in the rabbit, the injection of 80 mg/kg human glycoprotein fraction VI increased the total drug concentration in serum, decreased the unbound drug concentration and ΔQRS duration (Fig. V-2).

The data demonstrate that when the serum protein binding of a drug is altered, the pharmacological response parallels the change in the unbound drug concentration, not the total drug concentration in serum. Whenever total drug concentration is used to assess the pharmacological effect, the serum protein binding should always be considered.

For a drug with concentration-dependent serum protein binding, the unbound fraction in the sinusoids may be lower than in the arterial blood. For given values of blood flow, intrinsic clearance, and unbound fraction in the arterial blood, a drug with concentration-dependent serum protein binding has a lower clearance than a drug with linear serum protein binding. The discrepancy in the prediction of clearance is most dramatic when: the intrinsic clearance is high; the unbound drug concentration is much greater than the dissociation equilibrium constant of the drug-protein complex; there is no additional linear binding in plasma; partitioning to the red blood cells is absent; and the unbound fraction is small (Figs. VI-1, VI-2, and VI-4).

At low steady-state concentrations of S-disopyramide, the injection of human glycoprotein increased the serum protein binding of S-disopyramide and decreased hepatic blood clearance (Fig. VII-1). When the observed hepatic blood clearance was compared to the measured indocyanine green clearance, used to estimate hepatic blood flow, the relationship between the hepatic clearance and the protein binding was consistent with the prediction based on the "well-stirred" and "parallel tube" models.

Appendix

Part A.

In the "well-stirred" model, drug distribution inside the sinusoid is assumed to be homogenous and the drug concentration in the sinusoid is same as the drug concentration leaving the sinusoid. The rate of drug elimination can be expressed as:

$$Cl_l \cdot Cu_v = Q \cdot (C_B - C_{Bv}) \quad . \qquad \text{(Eq. A-1)}$$

Substituing Eqs. VI-4 and VI-5 into Eq. A-1, one obtains:

$$Cl_l \cdot Cu_v = Q \cdot \left(\frac{Cu}{\alpha_B} - \frac{Cu_v}{\alpha_{Bv}}\right) \quad . \qquad \text{(Eq. A-2)}$$

Rearranging Eq. A-2:

$$\frac{Cu}{Cu_v} = \frac{\alpha_B \cdot Cl_l}{Q} + \frac{\alpha_B}{\alpha_{Bv}} \quad . \qquad \text{(Eq. A-3)}$$

Assuming the partition coefficient into blood cells, λ, is equal to 0, Eqs. VI-7 and VI-8 are simplified to:

$$\alpha = \alpha_B \cdot (1 - H) \qquad \text{(Eq. A-4)}$$

$$\alpha_v = \alpha_{Bv} \cdot (1 - H) \quad . \qquad \text{(Eq. A-5)}$$

Substituting Eqs. A-4 and A-5 into Eq. A-3:

$$\frac{Cu}{Cu_v} = \frac{\alpha \cdot Cl_l}{Q \cdot (1-H)} + \frac{\alpha}{\alpha_v} \quad . \qquad \text{(Eq. A-6)}$$

When the unbound drug concentration is much greater than the dissociation constant, Eqs. VI-2 and VI-3 can be simplified:

$$\alpha = \frac{Cu}{Cu + Pt} \qquad \text{(Eq. A-7)}$$

$$\alpha_v = \frac{Cu_v}{Cu_v + Pt} \quad . \qquad \text{(Eq. A-8)}$$

Rearrangement of Eqs. A-7 and A-8 yields:

$$Cu = \frac{Pt}{\frac{1}{\alpha} - 1} \qquad \text{(Eq. A-9)}$$

$$Cu_v = \frac{Pt}{\frac{1}{\alpha_v} - 1} \quad . \qquad \text{(Eq. A-10)}$$

Substituting Eqs. A-9 and A-10 into Eq. A-6, gives:

$$\frac{Pt/(1/\alpha - 1)}{Pt/(1/\alpha_v - 1)} = \frac{\alpha \cdot Cl_l}{Q \cdot (1-H)} + \frac{\alpha}{\alpha_v} \quad . \qquad \text{(Eq. A-11)}$$

By cancelling the common terms and rearrangement, Eq. A-11 is simplified to:

$$\alpha_v = \frac{\alpha}{1 + \frac{Cl_l \cdot (1-\alpha)}{Q \cdot (1-H)}} \quad . \qquad \text{(Eq. A-12)}$$

Combining Eqs. VI-1 and A-5 gives:

$$Cl_{HB} = \frac{Q \cdot \alpha_v \cdot Cl_l}{Q \cdot (1-H) + \alpha_v \cdot Cl_l} \quad . \qquad \text{(Eq. A-13)}$$

Finally, combining Eq. A-12 and Eq. A-13 results in an equation for the extreme case of concentration-dependent binding of drugs:

$$Cl_{HB} = \frac{Q \cdot \alpha \cdot Cl_l}{Q \cdot (1-H) + Cl_l} \quad . \qquad \text{(Eq. VI-9)}$$

Part B.

Using the same assumptions as in Part A, that the unbound drug concentration is much greater than the dissociation constant and λ is equal to 0, Eqs. VI-11 and VI-12 can be rewritten as follows:

$$\frac{Cu_x}{C_{Bx}} = \frac{\alpha}{1 - H} \qquad \text{(Eq. A-14)}$$

$$\alpha_x = \frac{Cu_x}{Cu_x + Pt} \quad . \qquad \text{(Eq. A-15)}$$

Substituting Eq. A-15 into Eq. A-14 gives:

$$Cu_x + Pt = \frac{C_{Bx}}{1 - H} \quad . \qquad \text{(Eq. A-16)}$$

Taking the derivative on both sides of the equation with respect to C_{Bx}, the relationship is:

$$\frac{dCu_x}{dC_{Bx}} = \frac{1}{1 - H} \quad . \qquad \text{(Eq. A-17)}$$

Equation VI-10 which describes the rate of drug elimination based on the "parallel tube" model, can now be solved directly by incorporating Eq. A-17:

$$Q \cdot (1-H)\, dCu_x = -Cu_x \cdot \left(\frac{Cl_l}{L}\right) dx \quad . \qquad \text{(Eq. A-18)}$$

Integrating from 0 to L, one obtains:

$$\frac{Cu_v}{Cu} = exp\ \left(- \frac{Cl_l}{Q \cdot (1-H)}\right) \quad . \qquad \text{(Eq. A-19)}$$

Substituting Eqs. A-9 and A-10 into Eq. A-19, gives:

$$\frac{Pt/(1/\alpha_v - 1)}{Pt/(1/\alpha - 1)} = exp\ \left(- \frac{Cl_l}{Q \cdot (1-H)}\right) \quad . \qquad \text{(Eq. A-20)}$$

After rearrangement, Eq. A-20 can be expressed as:

$$\frac{\alpha_v}{\alpha} = \frac{exp\ \left(- \frac{Cl_l}{Q \cdot (1-H)}\right)}{1 - \alpha + \alpha \cdot exp\ \left(- \frac{Cl_l}{Q \cdot (1-H)}\right)} \quad . \qquad \text{(Eq. A-21)}$$

Dividing Eq. A-19 by Eq. A-21, gives:

$$\frac{Cu_v/Cu}{\alpha_v/\alpha} = 1 - \alpha + \alpha \cdot exp\ (- \frac{Cl_i}{Q \cdot (1-H)}) \quad . \quad \text{(Eq. A-22)}$$

As drug is assumed not to partition into blood cells, the left side of the equation is equal to:

$$\frac{Cu_v/Cu}{\alpha_v/\alpha} = \frac{Cp_v}{Cp} = \frac{C_{Bv}}{C_B} \quad . \quad \text{(Eq. A-23)}$$

Combining Eqs. A-22, A-23 with Eq. VI-6, which comes from the definition of Cl_{HB} and mass balance, gives the final form of the equation which describes the extreme of the clearance-binding relationship for concentration-dependent binding:

$$Cl_{HB} = Q \cdot \alpha \cdot (1 - exp\ (- \frac{Cl_i}{Q \cdot (1-H)})) \quad . \quad \text{(Eq. VI-14)}$$

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