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The pharmacokinetics of prazosin

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THE PHARMACOKINETICS OF PRAZOSIN

by

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DISSERTATION

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ABSTRACT

Prazosin, a selective $alpha_1$-adrenoceptor antagonist, is a potent vasodilating agent used orally in the treatment of hypertension and congestive heart failure. Animal studies have shown that prazosin undergoes extensive hepatic metabolism (>90%). Prazosin pharmacokinetic characterization is incomplete as an intravenous dosage form for human use is unavailable. Analytical techniques for prazosin analysis are time consuming and require 2 to 4 ml of sample.

A direct-injection HPLC fluorescence prazosin assay was developed that is simple, rapid, sensitive, involves no extraction steps and requires only 0.2 ml of biological sample. The bioavailability and disposition of prazosin (1 mg/kg) was studied in four beagle dogs. The experimentally determined bioavailability (77%) agreed with the predicted value (73%). Additional dog studies revealed a dose-dependent bioavailability, as bioavailabilities following small oral doses (1 and 5 mg) were three-fold less than the bioavailability determined after 1 mg/kg.

Prazosin pharmacokinetics after oral dosing were evaluated in nine heart failure patients and five healthy controls. As compared to the controls, the heart failure

group exhibited decreases in the prazosin blood clearance divided by the availability and the rate constant for elimination and an increase in the area under the plasma concentration-time curve.

Time-dependent peaks were observed during isocratic chromatographic analysis of plasma and blood samples from heart failure and normal subjects. An HPLC fluorescence gradient assay was developed to elucidate all components present in the biological sample.

The effect of acute and chronic cimetidine therapy on prazosin pharmacokinetics was studied in six healthy young adults. A large intra- and inter-subject variability was apparent over the course of the study. No statistical difference was found when comparing prazosin pharmacokinetic parameters when administered alone, with acute or chronic cimetidine dosing, or 18 hours after the discontinuation of chronic cimetidine administration.

To
Bob and Charlene,
my parents,
for without their encouragement,
support, understanding and love,
this achievement would have remained
only a dream.

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GLOSSARY

5'-AMP	5'-adenosine monophosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
AUC_B	area under the blood concentration-time curve
AUC_P	area under the plasma concentration-time curve
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CHF	congestive heart failure
CL_B	blood clearance
CL_{int}	intrinsic hepatic clearance
CL_P	plasma clearance
C_{peak}	peak plasma concentration
CRF	chronic renal failure
CV	coefficient of variation
E	extraction ratio
F	absolute bioavailability
F_{exptl}	experimental bioavailability
F_{pred}	predicted bioavailability
f_u	fraction of the drug unbound in plasma
gi	gastrointestinal
HPLC	high performance liquid chromatography
HR	heart rate
IS	internal standard
iv	intravenous
NA	not available
NE	norepinephrine
NEFA	non-esterified free fatty acids
NMR	nuclear magnetic resonance
NR	not reported
NRF	normal renal function
P	prazosin
PANS	parasympathetic autonomic nervous system
po	oral
POB	phenoxybenzamine

PRA	plasma renin activity
PRZ	prazosin
P:WB	plasma:whole blood concentration ratio
R_f	relative displacement
r^2	coefficient of determination
t_{lag}	lag-time
TLC	thin layer chromatography
$t_{½_z}$	terminal half-life
UV	ultra-violet
Vd_β	apparent volume of distribution
V_{ss}	volume of distribution steady-state
α	free fraction
λ_z	terminal rate constant of elimination

INTRODUCTION

Prazosin is a specific $alpha_1$-adrenoceptor blocking agent that is very effective in dilating both arterial and venous vascular beds. This compound is used in the treatment of all grades of hypertension, and as an adjunct to therapy of chronic congestive heart failure. It is increasingly being used in the study of adrenergic receptors since prazosin has an affinity for blocking $alpha_1$-adrenoceptors but has no effect on $alpha_2$-adrenoceptors. It is essentially completely metabolized by the hepatic route in both animals and man.

In humans, the pharmacokinetics of prazosin have not been studied in detail, due in part to the lack of a commercially available intravenous dosage form. Animal pharmacokinetic studies have also been limited, partly due to the large biological sample required for the available analytical techniques. All metabolic studies have been performed in the rat or dog, making extrapolation to man difficult at best.

The purpose of my research is to provide essential data on the pharmacokinetics of prazosin. The following experimental goals were evolved to that end:

1. to develop an assay specific for prazosin that is easy to perform, requires small volumes of biological sample

and which will have the requisite sensitivity at all concentration ranges that may be encountered;

2. to determine the pharmacokinetics of prazosin in beagle dogs following oral and intravenous administration;

3. to determine if disease-induced changes in human physiology alter the disposition of prazosin when the drug is administered to individuals with chronic congestive heart failure;

4. to develop analytical methods for the isolation and separation of prazosin metabolic products;

5. to appraise the effect of a reduction in liver blood flow, brought about by an endogenously administered agent, on the disposition of prazosin.

The dissertation is composed of eight chapters. In Chapter I the physicochemical and pharmacological properties of prazosin are reviewed. Prazosin pharmacokinetics and the effects of disease states upon the disposition of the compound are reviewed in Chapter II. In Chapter III the development of a specific and sensitive HPLC assay for this agent is detailed. The disposition of prazosin in beagle dogs, as well as evidence of a dose-dependent bioavailability in this animal model, is presented in Chapter IV. The pharmacokinetics of prazosin following oral administration in congestive heart failure patients is compared to normal controls in Chapter V. A discussion of prazosin metabolism,

the description of a specific and sensitive gradient assay for suspected prazosin metabolites, and evidence for the presence of apparently active metabolites in the plasma of patients on prazosin therapy is described in Chapter VI. The effects of the concomitant administration of cimetidine and prazosin in normal subjects is detailed in Chapter VII. In Chapter VIII the data presented in this dissertation is summarized together with a listing of conclusions drawn from this work.

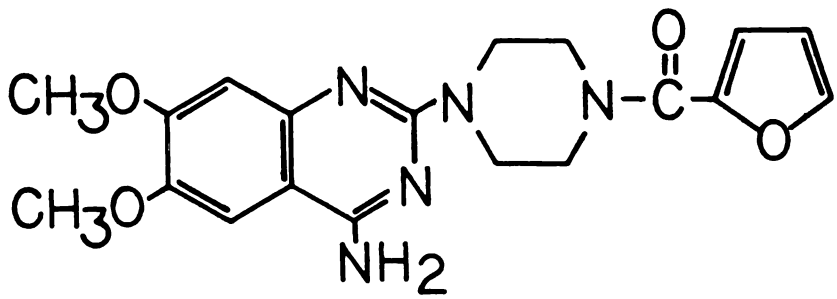
CHAPTER I

PHYSICOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF PRAZOSIN

A. Physicochemical Properties

Prazosin [I] is the prototypical agent of the new aminoquinazoline class of vasodilators. It was released for clinical use in the United States in 1976. It is a weak base, pK_a 6.5 in 1:1 ethanol/water, that is used therapeutically as the hydrochloride salt (Minipress,^{R} Peripress,^{R} Hypovase^R - Pfizer) (1,2). This compound is named chemically as a derivative of piperazine [1-(4-amino-6,7-dimethoxyquinazoline-4-(2-furoyl)-piperazine hydrochloride] or quinazoline [2-[4-(2-furoyl)-piperazin-1-yl]-4-amino-6,7-dimethoxyquinazoline hydrochloride]. Prazosin HCl has a molecular weight of 419.85 and an empirical formula of $C_{19}H_{22}ClN_5O_4$. Prazosin is a white crystalline powder with a melting point of 278 - 280^oC (1). It is slightly soluble in water and isotonic saline and very soluble in alcohol and ethyl acetate (1-3).

Prazosin hydrochloride should be stored below 30^oC. No degradation has been shown after storage in commercial containers under the following conditions: 12 weeks at 50^oC, 12 weeks at 37^oC at 75% relative humidity, 18 months at 37^oC and 36 months at 25^oC (3). Degradation due to temperature



PRAZOSIN [I]

or light has not been observed in the capsule dosage form (Minipress^{R}).

R_f values from silica-gel thin-layer chromatography are found in Table I-1, and infra-red and NMR data are located in Table I-2.

Table I-1. R_f values for prazosin in three different systems using silica-gel TLC plates. From Althius and Hess (4) and Taylor *et al*. (5).

SYSTEM	1	2	3
R_f	0.70	0.48	0.10

where (1) ethyl acetate - methanol - diethylamine (75:20:5)
(2) ethyl acetate - methanol (2:1)
(3) ethyl acetate - methanol - acetic acid (85:15:5)

Table I-2.

NMR spectra (deuterochloroform-DMSO-d_6) of prazosin. From Honkanen et al. (6).

δ	peak signal, proton count, coupling constants
3.38	(s, 14H)
6.57	(dd, 1H, J_1= 3.3 Hz, J_2= 1.6 Hz)
6.79	(s, 1H)
6.99	(d, 1H, J_1= 3.3 Hz)
7.01	(s, 2H)
7.43	(s, 1H)
7.74	(d, 1H, J_2= 1.6 Hz)

IR spectra of prazosin. From Honkanen et al. (6).

3319, 3226, 3077, 2857, 1634, 1597, 1481, 1468, 1425, 1280, 794, 763, 751, 721, 717, 675 cm^{-1}.

B. Adrenergic Pharmacology

The concept of neurohumoral transmission is derived from Elliot (7) who, in 1904, noted the similarity between the action of epinephrine and that of sympathetic nerve stimulation. This theory, which states that nerves transmit impulses across synapses and neuroeffector junctions by endogenous compounds known as neural humoral transmitters rather than by electrical action potentials, has been extensively studied and validated over the last 60 years. In 1921, Loewi (8) demonstrated the release of chemical transmitters in the perfused frog heart and in 1946 von Euler (9) demonstrated that mammalian sympathetic nerves contained norepinephrine. In the same year Hillarp (10) put forward the idea that the transmitter (norepinephrine) is released from terminal axon varicosities (11).

It is now accepted that following the synthesis and storage of norepinephrine in the vesicles in sympathetic nerve varicosities, an action potential will initiate the fusion of the vesicle with the plasma membrane and subsequent exocytosis of norepinephrine. The chemical transmitter crosses the synaptic cleft and binds to receptors in the post-synaptic membrane (effector cell) (see Fig. I-1). Norepinephrine and epinephrine, as well as other catecholamines, can cause either excitation or inhibition of smooth muscle contraction, depending on the site, the dose

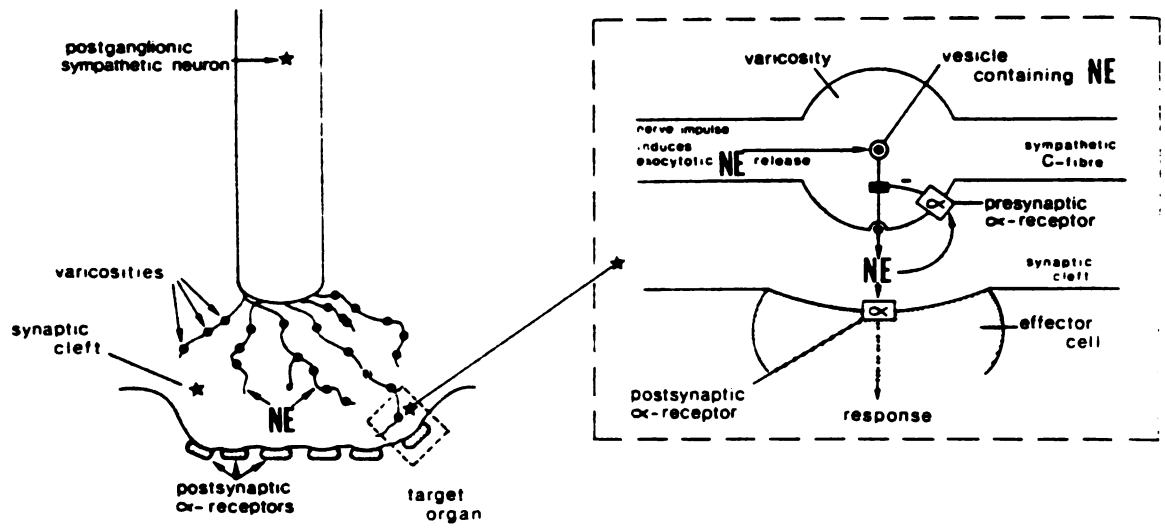


Figure I-1. Schematic representation of a noradrenergic synapse. Norepinephrine (NE) is released from the varicosities as a result of nerve activity. After passage across the synaptic cleft, the neurotransmitter norepinephrine stimulates postsynaptic α-adrenoceptors located on the cell membrane of the target organ, thus giving rise to a physiological effect. Stimulation of presynaptic α-adrenoceptors on the membrane of the varicosities (see insert) causes an inhibition of the amount of transmitter norepinephrine released per nerve impulse. [Adapted from Timmermans & VanZweiten (12)]

and the particular compound chosen (13).

In 1948, Ahlquist (14) tested a series of six catecholamines on a large variety of organs and systems from several species. He found the compounds to have one rank order of potency for vasoconstriction, pupil dilation and inhibition of the gut and an entirely different rank order when observing vasodilation and myocardial stimulation. Thus, there appeared to be two types of adrenotropic receptors which could not be classified as excitatory or inhibitory, as each type may have either action depending upon where it is found. Subsequently, these two receptor types were termed alpha adrenotropic and beta receptors (14).

Powell and Slater (15) in 1958 provided the experimental validation of this two receptor theory. They showed that an analog of isoproterenol, dichloroisoproterenol, selectively blocked the "inhibitory" effect of epinephrine and isoproterenol on blood vessels, bronchi and uterus, but had little to no effect on "excitatory" responses. The physiologic effects associated with stimulating the adrenergic nerves to various organs and effector cells are shown in Table I-3. The relative potency of a series of sympathomimetic amines on fatty acid metabolism, cardiac stimulation, bronchodilation and vasodepression led Lands and coworkers (16) to propose that beta receptors could be divided into two subtypes: $beta_1$ and $beta_2$. Today it is generally accepted that $beta_1$ receptors predominate in cardiac tissue

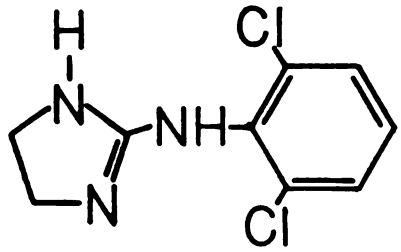
Table I-3. Responses of effector organs on stimulation of sympathetic (SANS) nerves. [Adapted from (6)]

EFFECTOR ORGAN	RECEPTOR TYPE	RESPONSE
Eye		
Radial muscle, iris	α	contraction (mydriasis)
Ciliary muscle	β	relaxation for vision
HEART		
SA node	β_1	increase in heart rate
Atria	β_1	increase in contractility, conduction velocity
AV node, His-Purkinje	β_1	increase in automaticity, conduction velocity
Ventricles	β_1	increase in contractility, automaticity, rate of idioventricular pacemaker
ARTERIES		
Coronary, skeletal muscle	α, β_2	constriction, dilation
Skin, mucosa	α	constriction
Cerebral	α	constriction (slight)
Pulmonary	α, β_2	constriction, dilation
Abdominal viscera, renal	α, β_2	constriction, dilation
Salivary glands	α	constriction
VEINS (systemic)	α, β_2	constriction, dilation
LUNG (bronchial muscle)	β_2	relaxation
STOMACH/INTESTINE		
Motility	α_2, β_2	decrease
Sphincter	α	contraction (usually)
Secretion		inhibition (?)
KIDNEY	β_2	renin secretion
URINARY BLADDER		
detrusor	β	relaxation (usually)
trigone, sphincter	α	contraction
LIVER	α, β_2	glycogenolysis, gluconeogenesis
SKIN		
pilomotor muscle	α	contraction
sweat glands	α	localized secretion

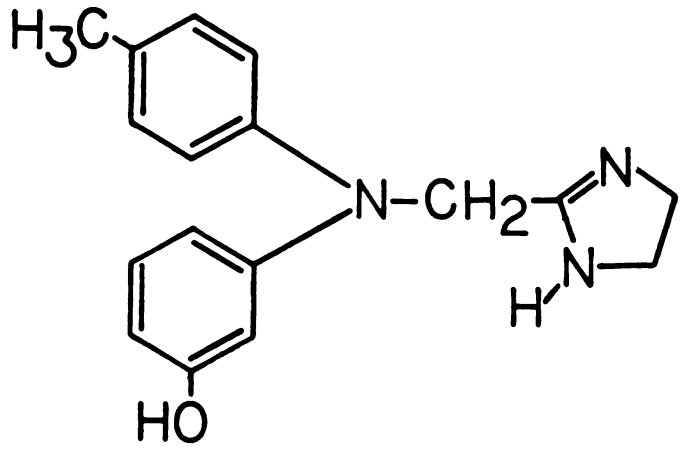
and the $beta_2$ receptors are preponderant in smooth muscle and gland cells, although different tissues may possess both subtypes in variable amounts.

The existence of different alpha adrenoceptors was proposed from work with clonidine [II] and classic alpha-receptor blocking agents (phentolamine [III], yohimbine [IV] and tolazoline [V]) on central nervous system (CNS) receptors (17), and from experiments on peripheral pre- and post-synaptic receptors (18,19). Since clonidine-induced sedation appeared to be mediated by the activation of alpha-adrenoceptors different from peripheral adrenoceptors, Delbarre and Schmitt (20) first suggested that the different alpha-adrenoceptors be identified as $alpha_1$ and $alpha_2$. This nomenclature had support from data obtained from preliminary studies of norepinephrine release/overflow mechanisms. It was thought that presynaptic regulation of norepinephrine release through a negative feedback system involved alpha-adrenoceptors. Further, when the potency of phenoxybenzamine in blocking both pre- and post-synaptic receptors was studied, it was found that a 30-fold less concentration was required for blockade of the post-synaptic receptor compared to that concentration required to enhance transmitter release during nerve stimulation (pre-synaptic receptor).

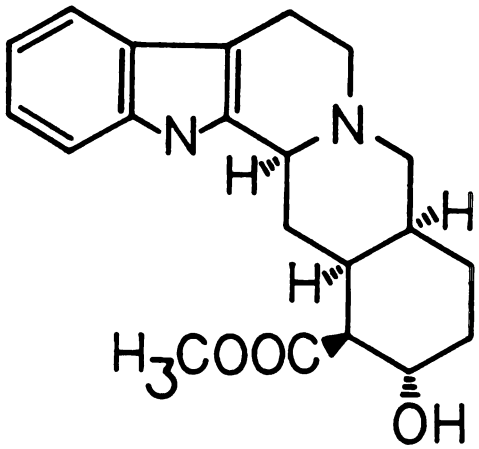
Berthelson and Pettinger (21), in their review of evidence related to a functional classification of alpha-



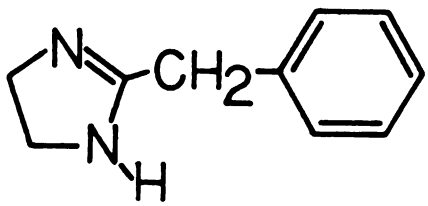
Clonidine [II]



Phentolamine [III]



Yohimbine [IV]



Tolazoline [V]

receptors, proposed a classification in which alpha$_1$ receptors are of the vascular smooth muscle type, and alpha$_2$ receptors are of the type found: a) on the sympathetic neuron terminal inhibitory to norepinephrine release, b) in the CNS inhibitory to the sympathetic nervous system, and c) on melanocytes inhibitory to dispersion of granules. Thus, alpha$_1$ receptors were thought to be postjunctional to the synapse and the alpha$_2$ receptors prejunctional. It now seems reasonable to assume that alpha$_1$ and alpha$_2$ receptors are general subtypes, occurring in many tissues, on neurones as well as non-neuronal cells, and should be defined not by location, but by pharmacology [i.e., their different affinities for drugs (22)]. This current concept is further supported by the fact that the alpha-adrenergic receptor inhibitory to melanocyte stimulating hormone-induced darkening of the frog skin behaves as though it is of the alpha$_2$ subtype even though it is located postsynaptically (22). Also, in large veins, and possibly in resistance vessels, the postjunctional vascular smooth muscle cell also contains alpha$_2$-adrenoceptors that can trigger the contractile process (23,24).

C. Prazosin Pharmacology

Prazosin is presently thought to exert its hypotensive effect by a functional inhibition of alpha$_1$-adrenoceptors

(25) with subsequent relaxation of the peripheral arterioles. Prazosin is the prototype of the new amino-quinazoline class of vasodilators, and as such, extensive studies have been undertaken to determine its exact mechanism of action. The mechanism described above has not always been the suspected or proposed action of this compound.

1. Development (History)

Prazosin was apparently developed from a specific design strategy, with the focus on increasing levels of cyclic adenosine monophosphate (cAMP). A number of studies had implicated cAMP as a mediator in smooth muscle relaxation. It became apparent that two enzymes, adenyl cyclase and phosphodiesterase, regulated cAMP concentrations. Adenyl cyclase increases the production of cAMP from adenosine triphosphate (ATP), and phosphodiesterase degrades cAMP to 5'-adenosine monophosphate (5'-AMP). Thus, it was thought that if intracellular levels of cAMP could be increased, this would result in the relaxation of smooth muscle with a subsequent fall in blood pressure. An increase in adenyl cyclase can be stimulated by phosphodiesterase inhibitors, beta-adrenergic agonists or alpha-adrenergic antagonists.

The inhibition of phosphodiesterase was thought to be the most promising mechanism (26), due to general

involvement of other sympathetic pathways following adrenergic receptor blockade. Also, a preliminary report indicated a decrease of responsiveness of adenyl cyclase following beta receptor activation in hypertensive rats versus normal rats (27). Further, increased concentrations of cGMP (found in many tissues with cAMP) had been shown to decrease the rate and force of contraction of the heart. It was concluded that an agent that would inhibit cAMP and cGMP breakdown would cause vasodilation without significant cardiac stimulation.

A number of structures with similarities to papaverine and theophylline, smooth muscle relaxants that inhibited phosphodiesterase, were used as prototypes. Structure activity relationships indicated that a compound ("structural hybrid") with the basic structure seen in Fig. I-2 would provide the greatest antihypertensive activity. A number of analogs were synthesized and prazosin was selected for clinical development on the basis of its relatively more favorable overall activity profile (28). Prazosin was shown to effectively inhibit the hydrolysis of cAMP and cGMP, and also to be 3 times more potent than theophylline and 24 times more potent than hydralazine in the dose-dependent inhibition of phosphodiesterase isolated from rat aorta. Thus, prazosin was entered into clinical trials with the anticipation that it would lower blood pressure, without increasing heart rate, by increasing intracellular concen-

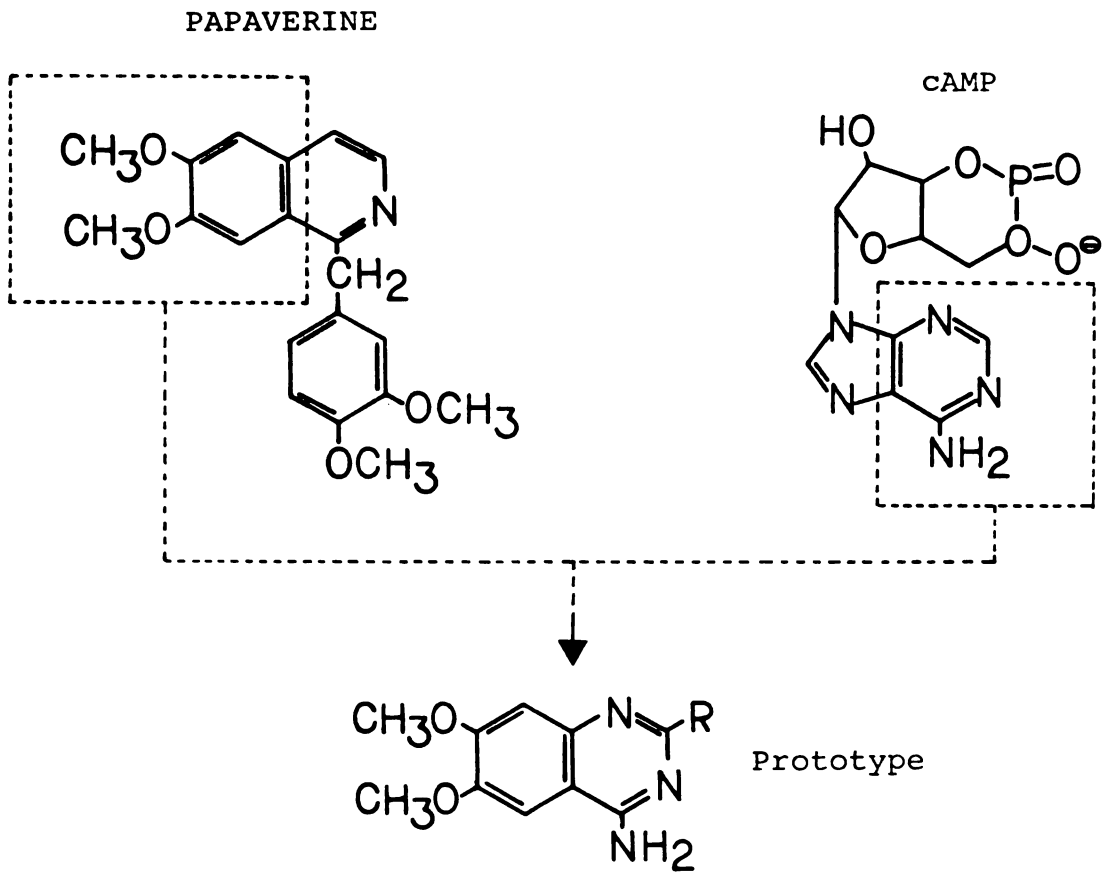


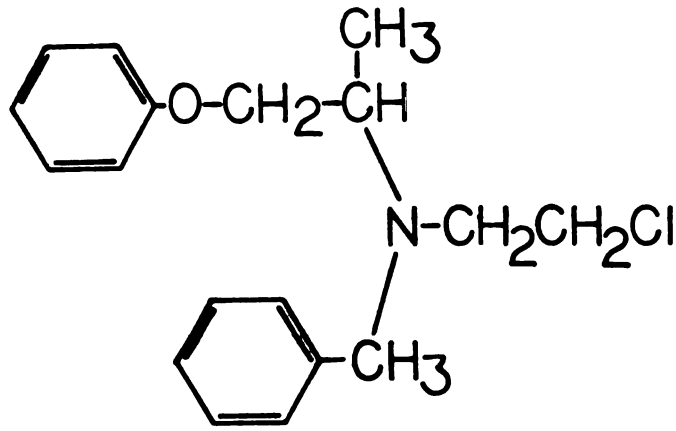
Figure I-2. Structural rationale for the synthesis of the prazosin prototype.

trations of cAMP in the vasculature and cGMP in the heart. The mechanism of action of prazosin was studied by Constantine et al. (29). They reported that the hypotensive effect was a direct result of peripheral vasodilation from two components of prazosin action: 1) direct smooth muscle relaxation, and 2) interference with peripheral sympathetic function by the drug interacting atypically at a site distal to the alpha receptor. Although the direct effect was believed to be the dominant action in lowering blood pressure, prazosin was thought to bring about a functional alpha-adrenoceptor blockade unlike that caused by traditional alpha-blocking agents.

2. Alpha-adrenoceptor Blocking Effect

The single most important study detailing the mechanism of action of prazosin was performed by Cambridge et al. (30) who compared the affinity of prazosin and phenoxybenzamine [VI] for pre- and post-synaptic alpha-adrenoceptors. Prior to a discussion of the results of that study, a brief explanation of presystemic regulation of catecholamine release is necessary.

When interpreting experimental evidence concerning norepinephrine release, it is important to distinguish transmitter release (from the presynaptic neurons) from transmitter overflow (out of the synaptic cleft). As shown



Phenoxybenzamine [VI]

in Figure I-3, release refers to the actual output of the transmitter following nerve stimulation, whereas overflow is an increase in norepinephrine concentration above resting levels. The resting levels are collected experimentally in the venous effluent of a perfused organ or in the bathing fluid surrounding an isolated organ preparation (31). The main sites of loss of released norepinephrine are: a) recapture of the released transmitter through neuronal uptake; b) extraneuronal uptake; c) metabolizing enzymes; and d) receptors (binding sites). Under normal conditions transmitter overflow is an underestimate of the total amount of norepinephrine released by nerve stimulation (Fig. I-3A). When one or several sites of loss are inhibited, overflow will be increased which is not secondary to changes in release (Fig. I-3B). Under these conditions, the increase in overflow is due solely to the blockade of a site(s) of loss. An increase in overflow can also be obtained by an increase in release (regardless of loss inhibition) (Fig. I-3C). Classic alpha-adrenoceptor blocking agents like phenoxybenzamine and phentolamine increase the overflow of norepinephrine following nerve stimulation. Iverson (32), Langer (33) and Starke *et al*. (34) discovered that in addition to inhibition of neuronal and extraneuronal uptake of norepinephrine, phenoxybenzamine increases the output of norepinephrine elicited by nerve stimulation. These results led to the hypothesis of a presynaptic regulation of norepinephrine release through a negative feedback mechanism mediated by

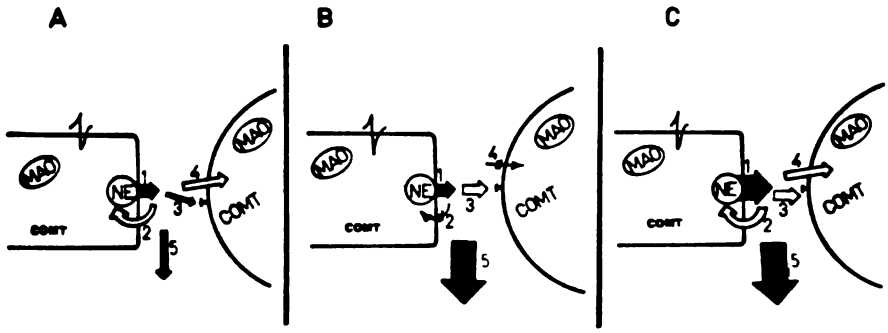
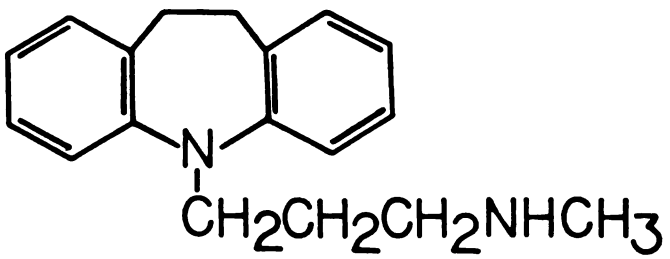


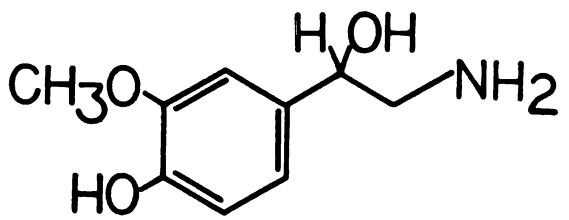
Figure I-3. Schematic representation of transmitter release and transmitter overflow during nerve stimulation. (A) Normal; (B) increase in overflow due to inhibition of sites of loss, no change in transmitter release; (C) increase in overflow due to an actual increase in transmitter release, sites of loss unaffected. 1) Total amount of transmitter released by nerve stimulation; 2) norepinephrine recaptured by neuronal uptake, subsequently deaminated or stored in the vesicles; 3) fraction of the transmitter released available for activation of the receptors of the effector organ; 4) norepinephrine taken up at extraneuronal sites, subsequently metabolized mostly by COMT; 5) overflow: norepinephrine collected during and after the period of nerve stimulation. (NE - norepinephrine; MAO - monoamine oxidase; COMT - catechol-o-methyltransferase) [From Langer (31)]

alpha-adrenoceptors. It was proposed that following nerve stimulation, a threshold concentration of norepinephrine released into the synaptic cleft would bind to alpha receptors and activate the feedback mechanism. This theory was compatible with the knowledge that alpha agonists inhibit transmitter release by stimulation and alpha antagonists enhance norepinephrine release. Also, the feedback mechanism would operate more effectively when the quantity of transmitter released by each impulse is high. Thus, when neuronal uptake is impaired, presystemic inhibition of transmitter release would be enhanced because a greater concentration of norepinephrine is available for the activation of presystemic alpha-adrenoceptors.

Cambridge et al. (30) estimated the affinity of prazosin and phenoxybenzamine for presynaptic alpha-adrenoceptors by measuring the stimulation-induced overflow of ^{3}H-norepinephrine. The affinity for post-synaptic sites was estimated by measuring changes in tension following nerve stimulation in isolated artery preparations. Since phenoxybenzamine blocks both neuronal and extraneuronal uptake, prazosin and phenoxybenzamine were studied in the presence and absence of uptake inhibitors (desipramine [VII] and normetanephrine [VIII]). Phenoxybenzamine caused a significant increase in the ^{3}H-norepinephrine overflow, whereas the overflow following prazosin did not deviate from control values (Fig. I-4). Both compounds caused significant con-



Desipramine [VII]



Normetanephrine [VIII]

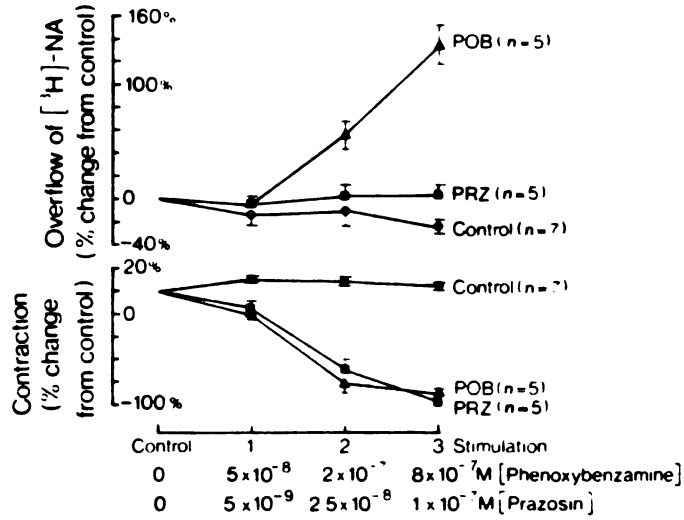
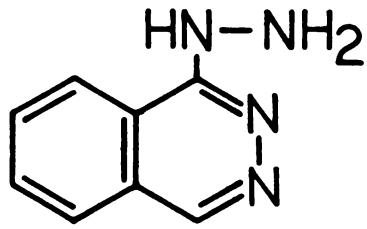


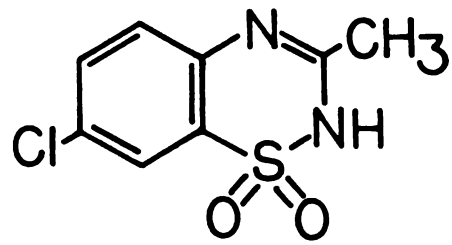
Figure I-4. Effect of prazosin and phenoxybenzamine on contraction and ^{3}H-NE overflow in rabbit pulmonary artery after blockade of uptake. The control stimulation is the mean of 2 responses. [From Cambridge et al. (30)]

traction of the isolated artery preparation (no change in control). This study was the first to show that prazosin had affinity for post-synaptic receptors, but little to no affinity for presynaptic sites.

The mechanism and effect of prazosin binding exclusively to post-synaptic alpha-adrenoceptors has been studied extensively since the report of Cambridge and coworkers (30). It has been clearly demonstrated (35-39) that unlike the vasodilators hydralazine [IX] and diazoxide [X], which act directly on the peripheral vasculature, prazosin acts as an alpha-adrenoceptor blocking agent. In 1979, Oates (40) reported a highly significant correlation between the degree of alpha blockade afforded by prazosin and the hypotensive response in anesthetized rats, such that it is evident that a close relationship exists between the two. Prazosin was also shown to inhibit central alpha-adrenoceptors to about the same degree as it did peripheral alpha-adrenoceptors (41), although this is not thought to contribute to its hypotensive activity. The lack of antagonism to the sympathetic discharge elicited by central stimulation (hypoglycemia) or the responses mediated through beta adrenergic receptors was determined by Buzzeo et al. (42) and Patel et al. (43). The determination and discovery of prazosin's mechanism of action coincided with the elucidation of $alpha_1$- and $alpha_2$-adrenoceptors. Prazosin is now considered one of the "classic" $alpha_1$ blocking



Hydralazine [IX]



Diazoxide [X]

agents and, outside of its therapeutic use, has been used extensively in receptor studies. It is currently being used to determine the locations of $alpha_1$ and $alpha_2$ receptor sites (44), and to study the effect of age on the responsiveness of vascular alpha-adrenoceptors in man (45).

3. Summary

Prazosin is a selective post-synaptic $alpha_1$-adrenoceptor blocking agent that causes the reversal of pressor responses to epinephrine and blocks pressor responses to norepinephrine without causing enhanced neural release of norepinephrine. As prazosin has no apparent effect on $alpha_2$-adrenoceptors, norepinephrine is allowed to exert negative feedback control of its own release, thus decreasing the cardiac stimulation that would follow non-selective alpha-adrenoceptor blockade. By the nature of its blocking properties, prazosin reduces tone in both resistance and vascular beds, thus decreasing total peripheral resistance, venous return and cardiac output.

D. Therapeutics

The major determinants in maintaining blood pressure are the total peripheral resistance (including blood volume) and the rate and force of cardiac contraction (see Fig. I-5). Any event that increases the cardiac output or peripheral resistance will increase blood pressure. And likewise, any agent that decreases these functions will decrease blood pressure, and, may be useful in the treatment of hypertension. Since alpha-adrenoceptors have been shown to play an important role in blood pressure control, a drug that would selectively inhibit these actions would have an important place in the management of different forms of hypertension (46)

1. Prazosin Therapy in Hypertension

Therapeutic trials conducted throughout the world have demonstrated the efficacy of prazosin in reducing all grades of hypertension (47-50). Prazosin is effective when used alone in cases of mild hypertension, and in some cases of moderate severity, and thus may be useful as a "first-line" agent. The efficacy of prazosin is increased by the addition of diuretics (51-55) or beta-adrenergic blocking agents (56-61), and the drug has its widest application when used in conjunction with other agents. In patients who cannot

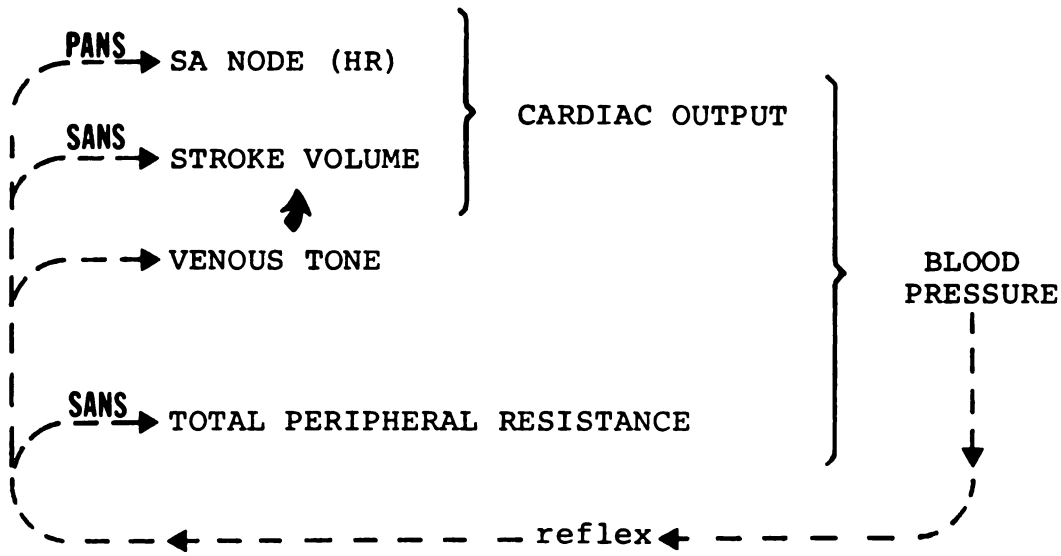


Figure I-5. Major determinants of blood pressure control. In general, the sympathetic (SANS) and the parasympathetic (PANS) autonomic nervous systems are viewed as physiological antagonists. When one system inhibits a certain function the other usually augments that function, although rarely to the same degree. The control of blood pressure is probably entirely due to sympathetic control of peripheral resistance (arteriolar resistance), as cholinergic vasodilation (PANS) at these sites is of questionable physiological significance. The identification of PANS and SANS reflex pathways in this figure indicates which nervous system possesses the greatest physiologic control for each blood pressure determinant. [(HR)-heart rate]

tolerate either diuretics, because of gout or hyperglycemia, or beta-blockers, because of asthma or cardiac failure (62), prazosin may be effective as the sole agent. The long-term efficacy of prazosin has been well studied and, unlike many other antihypertensive compounds, there has been no detectable tolerance to its peripheral resistance lowering effects (50,63,64).

2. Prazosin Therapy in Congestive Heart Failure

In 1977, Awan et al. (65) reported that prazosin produced a decline in forearm venous tone with a concomitant reduction in forearm vascular resistance. This work indicated that prazosin may be of benefit in the relief of pulmonary congestion and the elevation of cardiac output in patients with congestive heart failure. Subsequent work in that laboratory (66-69) demonstrated that prazosin possessed balanced dilator actions on the systemic arterial and venous beds, and that this compound was effective in the ambulatory management of chronic congestive heart failure. These findings were corroborated independently by other investigators (70,71). In 1979, hemodynamic and clinical tachyphylaxis of the prazosin-induced effect on cardiac output in cardiac failure patients was reported (72). The initial increase in cardiac index and the decreases in systemic vascular resistance and left ventricular filling pressure were transient

and serial administration of the drug could not generate the same magnitude of change as did the first dose. Follow-up studies further questioned the use of prazosin in heart failure by indicating that the effects of prazosin on hemodynamics were variable in the resting state, but that hemodynamics appeared to be consistently improved during exercise (73-76). The most recent work in this area (77-83), taken with all of the previous data, now indicates that prazosin does have a significant place in the therapy of congestive heart failure. It is effective in reducing both preload and afterload, and despite a gradual reduction in resting hemodynamics, the continued improvement seen during exercise would indicate that prazosin produces a sustained and well-tolerated effect in active patients with congestive heart failure.

3. Other Uses

In patients with catecholamine-secreting tumors, pheochromocytoma, a moderate to severe hypertension generally results that is often inadequately controlled by combined therapy with diuretics, beta-blockers and sympatholytics. Wallace and Gill in 1978 (84) reported the diagnosis and control of blood pressure and heart rate with prazosin in a single patient with pheochromocytoma. Cubeddu et al. (85) recently reported the successful preoperative management of

three patients with unilateral adrenal pheochromocytoma by combining prazosin and the beta-blocker propranolol. The reported number of patients is small and the tumors involved secreted norepinephrine. The effectiveness of prazosin therapy in pheochromocytoma, and particularly in epinephrine secreting tumors, remains to be established. However, the initial results indicate that prazosin (either alone or with propranolol) appears to be an effective agent in controlling the hypertension due to pheochromocytoma.

Prazosin use has been reported in a single case of ergotamine-induced peripheral ischemia (86). Ergotamine may damage the capillary endothelium, producing vascular stasis, thrombosis and gangrene. Prazosin is thought to effectively reduce the vasoconstriction by blocking ergotamine stimulation of alpha receptors

Prazosin was also reported to provide clinical improvement in 10 of 20 patients with severe Raynaud's disease; and to be of added benefit in 8 patients with aortic regurgitation (88) and in 8 patients with severe mitral regurgitation (89). Although prazosin was apparently of benefit in these brief trials, considerable study remains to be performed.

E. Clinical Use and Dosing

In the control of hypertension, prazosin therapy should be initiated with a dose of 1 mg taken two to three times

daily (62,90). Doses should then be increased by titrating to the desired reduction in blood pressure. Doses up to 30 mg daily have been given, but doses above this have not been shown to provide added benefit. The therapeutic dosages most commonly employed range from 6 to 15 mg daily in divided doses (90). The benefit of adding a diuretic to the treatment regimen has been clearly shown, and appears to warrant consideration in cases of tachyphylaxis on higher prazosin dosages. A beta blocker would also appear to be of use. When adding a diuretic or another antihypertensive agent, the prazosin dose should be decreased initially and re-titrated.

Experience with successful long-term therapy in congestive heart failure is more limited and dosages are not as well defined as for hypertension. Current recommendations suggest an initial dose of 1 mg two or three times daily, increasing gradually to the appropriate final dose. Bertel et al. (83) restricted the maximum dose to 5 mg three times daily in their patients, whereas Awan and coworkers (82) found a successful range of 2 to 7 mg four times daily. From the typical initial dose of 1 mg three times a day, a dose based on the weight of the patient (50 µg/kg given every eight hours) was used by Harper et al. (91). In subjects of normal weight, this would be a dosing range of 2.5 to 4.5 mg three times daily, which agrees quite well with the previous dosage regimens. As in therapy for hyperten-

sives, maintenance therapy in congestive heart failure is best achieved by titrating the dose to a desired clinical effect.

The safe use of prazosin in pregnant women and children has not been well documented. Although prazosin has been safely and effectively used in children with congestive heart failure (92), administration to these two groups should be undertaken with extreme caution.

F. Toxicology

Prazosin appears to be well tolerated in both hypertensive and congestive heart failure patients. Severe side effects, as seen with other antihypertensive agents such as sexual dysfunction following clonidine or methyldopa administration, drug-induced lupus-like syndrome and myocardial stimulation secondary to hydralazine therapy, have not been reported with prazosin.

Side effects associated with chronic prazosin dosing are generally mild and rarely necessitate withdrawal of the drug (48,62). The most commonly reported side effects following the initiation of prazosin therapy are drowsiness, dizziness and headache (93-95). The most severe side effect is postural hypotension and, potentially, syncope (96-98). This severe orthostatic event has been reported only after the initial administration of the drug, and is known as "the

first-dose phenomenon." It has not been reported in the course of chronic treatment. Following acute dosing, vasodilation is pre-eminent on the venous return compared to the arteriolar bed (65), which may cause a reduction in cardiac output. On standing there is a marked fall in arterial pressure, further enhanced by the apparent lack of a reflex tachycardia, which precipitates the syncopal episode. A number of mechanisms for this effect have been proposed, but the hypothesis of Semplicini and coworkers (98) appears to have the most convincing experimental evidence. They found that the severity of the first-dose effect was more pronounced in cases of hypovolemia. When an acute expansion of the plasma volume was initiated, the orthostatic fall in blood pressure was reduced. A significant inverse correlation was found between the orthostatic fall in pressure and the plasma volume. Prazosin, after chronic administration, has been shown to expand the plasma volume (98-101) and it is this effect of the agent that is thought to reduce the orthostatic event. Clinically, the adverse effect is avoided by initiating therapy with a small dose, with the first dose taken at bedtime.

Most antihypertensive agents have the adverse effect of elevating the plasma renin concentration, which tends to negate, at least partially, the hypotensive effect. Plasma renin activity (PRA) was shown to be reduced by 62% in 9 hypertensive patients when treated for at least four weeks

with prazosin as the sole hypotensive agent (102). The effect of prazosin on PRA in hypertensives with low-, normal- and high-renin values was studied by Rosenthal et al. (103). They reported that in all six patients with high-renin activity, blood pressure and PRA was reduced significantly within two weeks after starting prazosin therapy. Blood pressure was reduced in all twelve of the normal-renin patients, but PRA was reduced significantly in only four. In two patients with low-renin activity changes in blood pressure and PRA were not apparent. These authors conclude that prazosin suppresses PRA in patients with high-renin values whose elevated blood pressure may be governed by an increased sympathetic drive and maintained by high cardiac output.

The effect of PRA was studied in 21 hypertensive patients before and after four weeks of prazosin therapy (2 mg three times daily) (104). All mean PRA values were reduced by approximately 25%.

A small transient increase in plasma volume [as reported previously (98-101)] was evident following two months of chronic prazosin therapy (105). The PRA also showed a small, transient increase, whereas plasma aldosterone concentrations were unchanged. This suggested that volume expansion was not the result of a prazosin-induced stimulation of the renin aldosterone system. This work was later supported by Barbieri et al. (106). They reported

that following chronic prazosin dosing (3 mg/day for three weeks) no significant change was observed in PRA or plasma aldosterone concentrations, although a slight increase in body weight was evident.

Rubin and Blaschke (107) reported a significant elevation in PRA four hours after an acute prazosin dose in normal subjects who had been standing for 30 minutes, four hours after an acute prazosin dose. They also reported that plasma norepinephrine concentrations rose significantly in both supine and standing positions. From these results they proposed that although post-synaptic alpha-adrenoceptor blockade was demonstrable in other species, it was unlikely to be the mechanism of action in man. This was a contradictory theory, based on minimal evidence, that did not alter the acceptance of what had been previously held as the mechanism of action for prazosin.

In 1982, Morgani et al. (108) presented exciting data that indicates that the increase in renin during systemic $alpha_1$-adrenoceptor blockade may be independent of the fall in blood pressure, and the juxtaglomerular $alpha_1$-adrenoceptors may participate in the regulation of renin release with an inhibitory action.

Other adverse effects to prazosin include dry mouth, diarrhea, weight gain, nausea, urinary frequency with urgency, urinary incontinence, nasal congestion and constipation (90,91). In the vast majority of cases the side

effects are of mild to moderate severity, and either disappear or are relatively easily tolerated with continued therapy (55,91,109,110).

CHAPTER II

PHARMACOKINETICS

A. Absorption and Bioavailability

1. Animal Studies

Taylor et al. (5) studied the absorption and excretion of prazosin by administering a 1 mg/kg oral dose (in aqueous solution) of ^{14}C-prazosin and the same dose by intravenous administration to rats (n=4) and dogs (n=2). In rats total recoveries of ^{14}C from combined excretions (urine and feces) were similar after oral and intravenous dosing (88.8 $\pm$ 3.7 and 87.0 $\pm$ 2.5 as % of dose, respectively). In dogs, there was a lower recovery of ^{14}C in the urine and feces following the oral dose (64.3%) compared to the intravenous dose (82.4%). The difference was reportedly due to either incomplete combustion during the radiochemical assay, an incomplete collection and/or a non-homogenous blending of the fecal sample. When ^{14}C-prazosin was administered orally to a bile fistula dog, 50% of the dose was recovered from the bile within 72 hours. Assuming that the fecal excretion pattern of prazosin is similar regardless of the route of administration, this figure represents about 75% of the amount excreted in the feces by non-cannulated dogs after an oral or intravenous dose. This data also indicated that, in

the dog, approximately 30% of the labelled oral dose excreted in the feces does not come from biliary secretion. Unfortunately, apparent errors in fecal collection and/or assay, and the lack of intravenous data in the bile fistula animal, precludes any further conclusions concerning prazosin absorption in dogs. Further investigation on prazosin absorption in dogs was performed by Rubin et al. (111) who reported that the bioavailability of prazosin in three conscious dogs was 38 ± 11%.

2. Human Studies

a. Normal subjects

Prazosin absorption in humans was first reported in 1976 by Wood et al. (112). From their study of ten normal subjects they reported a relatively large variation in the absorption rate of drug from a 5 mg tablet. The time of the peak plasma concentration ranged from 1 to 5 hours (two subjects at 1 hour, six at 2-3 hours, one at 4 hours and one at 5 hours). The variability in the peak time has been supported by other investigators (see Table II-1). Different pharmaceutical dosage forms have been developed: Minipress^{R} - capsules; Hypovase^{R} - tablets; and, Peripress^{R} - tablets. The variability in the rate of absorption, primarily due to inter-subject differences in absorption rate, may also be

Table II-1. Prazosin absorption parameters following oral administration to healthy subjects.

Author/year (ref)	# of subjects	dosage form	Dose (mg)	Mean peak conc (ng/ml)	Mean peak time (hrs)	F (%)
Wood et al.,1976 (112)	10	tab	5	23 ± 10[a]	2-3[b]	NR
Verbesselt et al.,1976 (115)	18	tab	2	23 ± 11[c]	1.5	NR
Simpson et al.,1977	10	NR[d]	5	27 ± 10	2-4[b]	NR
(116)	5	NR	3	32 ± 10	NR	NR
Hobbs et al.,1978	24	cap[e]	5	35.9 ± 17.3	2.2 ± 1.1	NR
(113)	24	cap[e]	5	39.1 ± 18.1	2.0 ± 1.0	NR
	21	soln	5	58.2 ± 16.8	0.7 ± 0.4	NR
Bateman et al.,1979 (117)	6	tab	1	5.6-13[b]	1.5-2[b]	56.9 ± 9.7
Jaillon et al.,1979 (118)	10	cap	5	NR	2.05	NR
Dynon et al.,1980	5	tab	0.5[f]	2.7	2.2	NR
(119)	5	tab	1.5	5.8	2.0	NR
Rubin et al.,[g]1981 (120)	7	NR	1	NR	NR	68 ± 17
Pitterman et al.,1981 (121)	5	cap	5	29.3 ± 6.6	1	NR

[a]Mean ± SD [b]range, mean value not reported [c]Cpeak reported here is the highest concentration of all samples at each sampling time. It is not the mean of all peak concentrations. [d]not reported [e]Total 5 mg dose is composed of two 2 mg capsules and one 1 mg capsule. [f]0.5 mg dose is one-half of a 1 mg tablet. [g]young subject group only

due in part to the dosage forms utilized in the different investigations. The extent of absorption appears equivalent regardless of whether dosing is by solution, tablet or capsule (114).

b. The effect of food on absorption

The influence of food on the rate and extent of absorption was studied by Verbesselt et al. (115) in 1976. Eighteen healthy volunteers received prazosin as a 2 mg oral tablet when fasting or immediately following a standard breakfast or lunch. Although Verbesselt et al. observed a large intra- and inter-subject variability in prazosin plasma concentrations, expressed as area under the plasma concentration-time curve (AUC), there was no clear overall effect of concomitant food administration upon the extent of absorption. The only trend observed was a difference at the 30 minute sample, as 13 out of 18 subjects (72%) had plasma concentrations greater than 10 ng/ml under fasting conditions, whereas with food, concentrations equal to or in excess of 10 ng/ml occurred in 14 out of 36 subjects (39%) following oral administration (115).

c. Absolute bioavailability

Although numerous studies with prazosin have been conducted, detailed information concerning disposition and absolute bioavailability[1] are lacking as an intravenous preparation was not available. To date, there have been only two studies on the absolute bioavailability of prazosin in normal subjects (see Table II-1). In 1979, Bateman et al. (117) administered prazosin hydrochloride (equivalent to 1 mg free base) both orally and intravenously to six healthy male volunteers. Following both iv and oral dosing, venous blood was sampled for 12 hours and urine was collected for 24 hours. The absolute bioavailability (F), expressed as a percentage, was calculated by comparing the areas under the oral and intravenous plasma concentration-time curves:

$$F = (AUC_{po} / AUC_{iv}) \times 100 \qquad \text{(II-1)}$$

These authors reported that the bioavailability ranged from 43.5 to 69.3% with a mean of 56.9 $\pm$ 9.7% (116).

In 1981, Rubin et al. (120) reported the absolute bioavailability of prazosin in seven young (22-32 years) and

[1]In this context, the absolute bioavailability of a drug is defined as the comparison of plasma level or urinary excretion data following non-intravenous (oral, intramuscular, etc.) administration to that following intravenous administration of a solution of the drug.

seven elderly (66-78 years) healthy subjects. Each subject was studied on two occasions at least one week apart. On one study day subjects received 1 mg prazosin orally three hours after a light breakfast. On the other study day the young subjects received 1 mg and the elderly subjects 0.5 mg of prazosin gluconate by rapid intravenous injection. The bioavailability was determined by comparing the dose-corrected areas under the plasma concentration-time curve:

$$F = \frac{Dose_{iv} \times AUC_{po}}{Dose_{po} \times AUC_{iv}} \times 100 \qquad \text{(II-2)}$$

The authors reported that the bioavailability in the elderly (48 $\pm$ 16%) was significantly lower that in the young subjects (68 $\pm$ 17%); that is, about 40% less unchanged drug reached the systemic circulation in the elderly as compared to the young. Individual values were not reported, but there does appear to be a relatively large variability in each group.

3. Effect of Disease States on Absorption and Bioavailability

a. Hypertension

Collins and Pek (122) in 1975 were the first to report prazosin pharmacokinetics in any subject group (normal or

disease state). Four fasting hypertensive subjects received prazosin as single 2 to 5 mg oral doses, and blood samples were taken serially for 7 to 24 hours. The mean peak concentration was obtained at 3.6 hours (range: 1.5 - 5 hours).

Only three other pharmacokinetic studies involving hypertensive patients have reported absorption parameters (see Table II-2). Graham et al. (98) reported a peak concentration (C_{peak}) of 14.9 ± 3.5 ng/ml and the time of the peak concentration (T_{peak}) of 1 to 2 hours in four patients given a 2 mg oral dose. The results from a single 2 mg dose administered orally (two 1 mg tablets) and intravenously in ten hypertensive patients (5 men and 5 women) were reported by Flouvat et al. (123). The mean peak plasma concentration following oral dosing was 18.7 ± 2.4 ng/ml. The T_{peak} was quite variable, ranging from 1.5 to 4 hours. The absolute bioavailability ranged from 34 to >100%, with a mean value of 62 ± 24 %.

A more complete pharmacokinetic study was performed by Grahnen et al. (124), who administered prazosin orally in 8 patients (4 men and 4 women) and intravenously in 4 of those patients. They reported that following the oral administration of 0.5 mg (one-half of a 1 mg tablet) the C_{peak} was 6.7 ± 2.9 ng/ml (range of 3.7 to 13 ng/ml) and the T_{peak} was 1.0 ± 0.5 hours (range: 0.5 to 2.0 hours). Prazosin was administered at the same amount by the iv route, and by comparing AUC they reported a mean bioavailability of 63 ± 13%

Table II-2. Prazosin absorption and bioavailability (F) parameters in patients with hypertension (with and without renal failure) or with congestive heart failure.

Author/year (ref)	# of subjects	dosage form	Dose (mg)	Mean peak conc (ng/ml)	Mean peak time (hrs)	F (%)
HYPERTENSION						
Collins & Pek, 1976 (122)	4	NR[a]	2-5	NR	3.6	NR
Graham et al.,1976 (98)	4	tab	2[b]	14.9 ± 8.6	1-2	NR
Flouvat et al.,1979 (123)	10	tab	2	NR	NR	62 ± 24
Grahnen et al.,1981 (124)	8	tab	0.5[c]	6.7 ± 2.9	1.0 ± 0.5	63 ± 13[d]
Chaignon et al., 1981 (125)	9	tab	2[b]	20.4 ± 5.1	2.7 ± 2.1	NR
HYPERTENSION WITH CHRONIC RENAL FAILURE						
Lowenthal et al.,1980 (126)	5	NR	1	NR	3[e]	NR
Chaignon et al.,1981 (125)	9	tab	2[b]	33.5 ± 11.1	1.2 ± 0.6	NR

Table II-2. (continued)

Author/year (ref)	# of subjects	dosage form	Dose (mg)	Mean peak conc (ng/ml)	Mean peak time (hrs)	F (%)
CONGESTIVE HEART FAILURE						
Jaillon et al., 1979 (118)	9	NR	2	NR	2.2	NR
Silke et al.,1981 (127)	8	NR	0.5[c]	6.2 ± 2.5	2[f]	NR
	8	NR	1	9.4 ± 7.1	2[f]	NR
	5	NR	2	20.3 ± 6.9	2[f]	NR
	5	NR	4	37.1 ± 15.4	2[f]	NR

[a] not reported

[b] as two 1 mg tablets

[c] one-half of a 1 mg tablet

[d] F determined in four patients

[e] samples taken at 0,1,3,5,8 and 10 hrs only

[f] samples taken at 0,0.5,1,2,4 and 8 hrs only

All values reported as Mean (± SD)

(range: 54 to 82%). The data, although illustrating significant variability, would indicate that the absorption of prazosin in hypertensives is similar to that found in normals.

b. Congestive Heart Failure (CHF)

Drug therapy in patients with cardiac disease may be complicated by disease-related variability in absorption kinetics (129), due either to changes in gastrointestinal motility, intraluminal gi pH or splanchnic blood flow (129,130). Prazosin is used in the treatment of CHF (see Chapter I, section D.2) and thus, it becomes of clinical importance to determine prazosin absorption and bioavailability parameters in this class of patients.

Jaillon et al. (118) reported that following the oral administration of prazosin to ten normal subjects (5 mg capsule) and to nine CHF patients (2 mg, dosage form unspecified), that the T_{peak} was similar between the two groups. The mean T_{peak} for the normal subjects was 2.1 hours, and in the CHF group it was 2.2 hours. Following the single oral dose the normal subjects did show a significantly lower dose-corrected AUC than did the CHF group (26.7 vs 56.4 ng.hr/ml per mg).

Silke et al. (127) determined the C_{peak} and T_{peak} in five patients with severe chronic congestive heart failure

following four oral doses (0.5, 1, 2 and 4 mg) of prazosin. They reported no difference in the time of peak concentration, which was consistently at the 2 hour sampling time. Unfortunately the authors chose to sample the blood at only 0.5, 1, 2, 4 and 8 hours post-dosing. This is an infrequent sampling schedule and the data reported should not be treated as an exact measure of T_{peak}. Dose-corrected mean peak concentrations following 0.5, 1, 2 and 4 mg orally were 76, 64, 85 and 63 ng/ml per mg, respectively. As in all previous studies on prazosin pharmacokinetics, the authors reported a considerable between-subject variability in plasma concentrations at all times after each prazosin dose.

Thus, while pharmacokinetic studies detailing the absorption of prazosin in CHF patients are few, we can reasonably conclude that the peak time appears to be the same as in normals. The work of Jaillon and coworkers (118) indicates that the dose-corrected AUC following oral prazosin is greater in CHF patients than in normals, but until an intravenous preparation becomes available, a definitive study (including clearance and absolute bioavailability) cannot be undertaken.

c. The effects of renal failure on absorption

Collins and Pek (122) reported that a patient with hypertension and renal impairment who received a single dose

of prazosin, had plasma concentrations that decreased too slowly during the study period such that a half-life could not be determined. They speculated that metabolism in the liver may have been affected in this patient. Stokes et al. (128) presented preliminary evidence for a greater fall in blood pressure in a group of patients with chronic renal failure than in a group with normal renal function. The authors suggested that this enhanced effect was due to an increase in the bioavailability of prazosin in patients with renal failure. A decrease in the metabolic activity in these patients would also appear to explain the greater fall in blood pressure in this group.

Hypertensive patients with and without normal renal function were studied by Lowenthal et al. (126). Seventeen patients were studied (5 with normal renal function and 12 with an impaired renal status); plasma was assayed for prazosin following single and repeated doses. Unfortunately little pharmacokinetic information was reported from this study. The authors state that the absorption of prazosin was not altered in the hypertensive patients with impaired renal function as similar peak concentrations and pharmacological effects in the two study groups would rule out a difference in drug absorption. In reviewing the data however, mean peak prazosin concentrations after a single oral dose and following low dose chronic therapy in hypertensive subjects with normal renal function was approximately two-

fold greater than peak concentrations in subjects with impaired renal function. Further, plasma samples from only 8 subjects, renal status unknown, were assayed for prazosin, and the plasma was infrequently sampled at 1, 3, 5, 8 and 10 hours post-dosing. The peak prazosin concentration has been reported to occur approximately 2 to 3 hours following oral dosing, and the sampling times from this protocol could not allow for an accurate determination of C_{peak} and T_{peak}. The conclusions drawn from this work are not supported by the study design or the results.

The pharmacokinetics of prazosin following the oral administration of 2 mg (two 1 mg tablets) was determined by Chaignon et al. (125), in two hypertensive groups - patients with concomitant chronic renal function and those with normal renal function. The mean C_{peak} was significantly higher (33.5 $\pm$ 11.6 vs 20.4 $\pm$ 5.0 ng/ml) and occurred considerably earlier (1.3 $\pm$ 0.7 vs 2.7 $\pm$ 1.0 hours) in the renal failure group than in hypertensive subjects with normal renal function. The total AUC was greatly increased in the renal failure group (206 $\pm$ 98 ng.hr/ml) as compared to the normal renal function group (117 $\pm$ 32 ng.hr/ml). These authors (125) concluded that the bioavailability of prazosin is altered in hypertensive patients with impaired renal function.

As will be discussed in the Metabolism and Elimination sections of this chapter, there appears to be no physiologic

mechanism that would alter prazosin pharmacokinetics in individuals with altered renal function since less than 5% of a prazosin dose is excreted unchanged in the urine. Care must be taken in extrapolating pharmacological effect data to absorption mechanisms, and obviously, more work is required in this area.

B. Distribution

Prazosin is a weak base and, as is the case for most basic compounds, it is relatively well distributed into the "tissues" of the body. Individual tissue distribution studies with prazosin are few, the data reported here is from work done at Pfizer Central Research, Groton, CT.

1. Animal autoradiography study

The available data (5) is from a study in one healthy male mongrel dog where ^{14}C-prazosin was given intravenously as a single 0.4 mg/kg dose. The animal was sacrificed 30 minutes after dosing, autopsied and selected tissues were removed for radioimmunoassay. Assuming that complete distribution has occurred, the results indicate that prazosin is found mostly in highly perfused tissue (see Fig. II-1).

Following the intravenous administration of 1 mg prazosin to three mongrel dogs, Rubin *et al.* (111) calculated

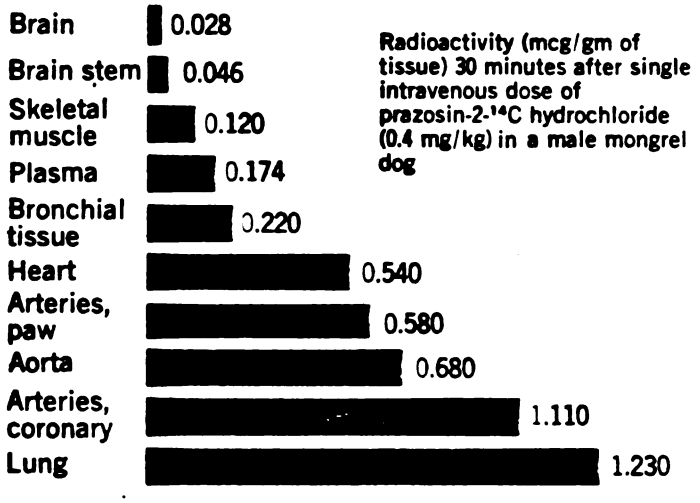


Figure II-1. Tissue distribution of ^{14}C-prazosin in a healthy mongrel dog. [From Hess (28)]

the volumes of distribution at steady-state (V_{ss}). The V_{ss} values in the three animals were 44.8, 35.6 and 65.5 liters, with a mean (± SD) of 48.6 ± 15.3 liters. This is a relatively large distributional volume, and the authors concluded that, as with many basic drugs, extensive tissue distribution occurs.

2. Distribution in humans

In man, the V_{ss} in normal subjects has been reported (as the mean) to be 42.2 and 48.8 liters by Bateman et al. (117) and Rubin et al. (120), respectively. Rubin and coworkers (120) also calculated the V_{ss} in healthy elderly male subjects, and it was found to be 69.2 liters. The mean apparent volume of distribution (Vd_{β}) in 24 healthy controls was reported by Hobbs et al. (113) to be 66 liters. In hypertensive patients with normal renal function, Flouvat and coworkers (123) reported a mean V_{ss} of approximately 39 liters, and Grahnen et al. (124) estimated a mean Vd_{β} of 48 liters. Although somewhat variable, this distributional data suggests that prazosin in humans, as in dogs, is extensively distributed to the tissues.

3. The protein binding of prazosin

In 1978, Hobbs et al. (113) determined by ultracentrifugation that prazosin is bound to human plasma proteins to the extent of 97%. The serum protein binding properties of prazosin were determined by the equilibrium dialysis method (131) using radiolabelled prazosin with a high specific activity. Concentrations studied were those reported previously as being present in the serum during therapy. The percentage of prazosin bound to serum proteins was 93.0, 92.8 and 89.7% at concentrations of 150, 50 and 20 ng/ml, respectively. Drugs which could potentially be co-administered with prazosin were tested for their possible effects on the serum protein binding of a 50 ng/ml prazosin sample (see Table II-3). Concentrations for the additional agents were chosen to be representative of plasma concentrations seen following therapeutic doses. Chlorpropamide at extremely high clinical concentrations significantly altered the serum protein binding of prazosin, but at a lower chlorpropamide concentration (still above the therapeutic range) there was no effect. The lack of a prazosin-dicumarol binding interaction confirmed earlier work by Crispino and DiCarlo (132).

In 1980, Rubin and Blaschke (133) reported the results of a more detailed study of prazosin binding. They performed equilibrium dialysis experiments to determine the

Table II-3. The serum protein binding of prazosin[a] and the influence of other therapeutic agents. From Hobbs & Twomey (131).

Added drug, concentration	percent bound ± SD
None	92.8 ± 1.6
Chlordiazepoxide, 70 µg/ml	90.7 ± 2.9
Chlorpropamide, 300 µg/ml	87.6 ± 1.6[b]
Chlorpropamide, 100 µg/ml	90.9 ± 0.7
Dicumarol, 100 µg/ml	90.2 ± 1.2
Methyldopa, 100 µg/ml	92.3 ± 1.5
Phenobarbital, 300 µg/ml	92.9 ± 0.9
Phenytoin, 100 µg/ml	92.4 ± 1.0
Polythiazide, 60 ng/ml	92.7 ± 1.0
Propranolol, 250 ng/ml	91.5 ± 1.4

[a] prazosin concentration, 50 ng/ml (Each value is the mean of 4 to 6 determinations.)
[b] statistically significant difference from control (t-test, multiple comparison test)

binding of ^{14}C-prazosin to albumin, to alpha$_1$-acid glycoprotein and to the plasma proteins of normal subjects and patients with cirrhosis, chronic renal failure or chronic heart failure. They reported a dissociation constant for prazosin binding to albumin of 3 X 10^{-5} M and to alpha$_1$-acid glycoprotein of 1.9 X 10^{-6} M, i.e., a 16-fold greater affinity for alpha$_1$-acid glycoprotein than for albumin. They also confirmed previous work (131) showing that propranolol, over a wide range of concentrations, did not change the percentage of free prazosin (free fraction) in human plasma. The free fraction of prazosin was significantly greater in patients with cirrhosis and in those with chronic renal failure, but not in congestive heart failure patients when compared to normals (see Table II-4). The investigators suggest that based on the greater range of prazosin free fraction in the patient groups, initial dosages should be prescribed with caution in subjects with these conditions.

Pitterman et al. (121), using blank plasma from each subject in their study, determined the protein binding of prazosin by equilbrium dialysis. They reported 94 $\pm$ 3 % of prazosin bound to plasma proteins.

Equilibrium dialysis measurements of prazosin protein binding in hypertensive patients were reported by Lowenthal et al. (126) and Grahnen et al. (124), as 92.5 and 97%, respectively. These values are consistent with those reported in normals.

Table II-4. The protein binding of prazosin in disease states. From Rubin & Blaschke (133). (Mean ± SD)

Patient population	% free	Range in % free	significance level compared to normal[a]
normal (n=14)	5.0 ± 0.7	4.2 ± 6.7	
cirrhosis (n=7)	6.4 ± 1.7	4.6 ± 9.2	$p < 0.05$
chronic renal failure (n=9)	7.7 ± 3.3	5.0 ± 15.0	$p < 0.05$
congestive heart failure (n=8)	6.4 ± 2.7	4.0 ± 13.0	$p > 0.05$

[a] unpaired t-test

The *in vivo* adminstration of heparin will initiate a cascade of biochemical events resulting in an increase in non-esterified free fatty acids (NEFA), which will displace prazosin from its binding sites on plasma proteins. Giacomini *et al.* (135) studied the *in vivo* effect of heparin on the plasma protein binding of prazosin, and found that if an inhibitor of this increase in NEFA is administered *in vivo*, *e.g.* paraxon, no effect on prazosin protein binding is seen. If heparin is added to plasma *in vitro*, NEFA are unaffected and there is no effect on the plasma protein binding of prazosin.

4. Binding to red blood cells

The plasma to whole blood concentration ratio (plasma:blood) for prazosin in man was reported by Grahnen *et al.* (124) to be 1.20 $\pm$ 0.05. The concentration range over which the samples were taken was not reported. Collins and Pek (122) added prazosin to the blood from an hypertensive patient *in vitro*; 10 minutes later the sample was centrifuged to separate plasma from red blood cells. The ratio of prazosin in plasma to that in the red cells was reported to be 1.6.

Rubin and Blaschke (133) added ^{14}C-prazosin, to achieve a concentration of approximately 30 ng/ml, to 20 ml of fresh blood treated to reduce clotting and maintained at 37^{o}C in a

shaking water bath. Samples were taken at 1, 5, 10, 15, 20, 30, 40, 50, 60, 90 and 120 minutes and assayed using a scintillation counter. Equilibrium between red cells and plasma was achieved in less than 60 seconds, and at equilibrium 20% of the prazosin in blood was associated with red cells.

C. Prazosin Metabolism

The metabolic profile of prazosin in humans has not been reported to date. Most of the information available comes from two published studies in animals. Numerous extrapolations and assumptions to humans are based on these animals studies, thus they will be reviewed here in some detail.

The first report detailing the metabolism of prazosin was by Hess (26) in 1974, which appeared again in 1975 (28). This report indicated that drug metabolism studies were undertaken to identify possible active metabolites and to select the appropriate animal model for long-term safety studies. This report was preliminary, and in 1977 Taylor et al. (5) published a more complete account of prazosin metabolism in rats and dogs.

Four rats received an intravenous dose of 1.0 mg/kg base equivalent of ^{14}C-prazosin, and a similar dose was given orally to four additional rats. To collect bile samples, the bile duct of one rat was cannulated and labelled

prazosin was given intraperitoneally in three doses over 24 hours. Two adult male beagles were dosed with 1.0 mg/kg of ^{14}C-prazosin orally as an aqueous solution, and then after a two week washout period these animals received the same dose intravenously. Labelled prazosin was also given orally to an adult beagle with a surgically prepared chronic biliary fistula. Urinary and fecal excretion of ^{14}C in these animals was described in Section A.1 of this chapter.

Using TLC and mass spectra comparisons with reference compounds, the isolation and identification of prazosin and two metabolites was accomplished (XI, XVI and XVII, see Figure II-2). Drug-related polar materials that did not migrate on TLC were treated with beta glucuronidase and sulphatase and then separated on TLC. Mass spectral data from these unknowns were compared to spectra of the 6- and 7-demethyl metabolites synthesized by Althius and Hess (26). Roughly 97% of radiolabelled compound from the dog and 92% of drug-related products from the rat could be identified (see Fig. II-3). O-demethylation and subsequent glucuronidation were reported to be the major metabolic pathways (see Fig. II-2).

A preliminary study with unlabelled prazosin in man has been mentioned in other publications by the Pfizer group (5,26,28). Chloroform extracts of basified human urine were separated by TLC and compared to similar extracts from dog bile. The authors (5) reported that the pattern of apparent

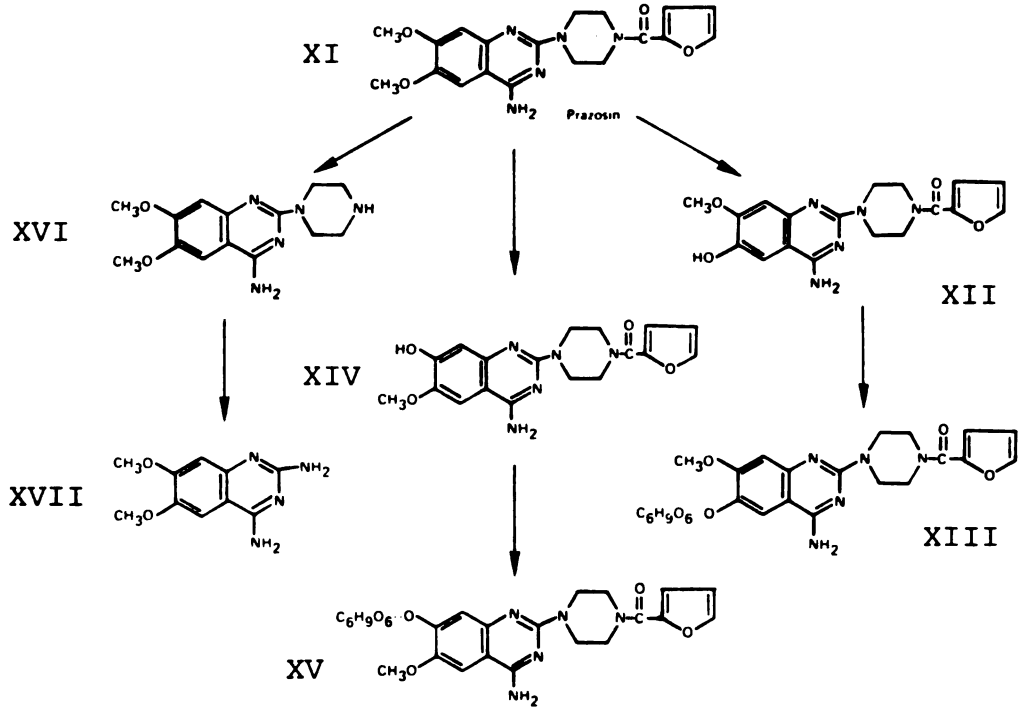


Figure II-2. Metabolic scheme of prazosin. [From Taylor et al. (5)]

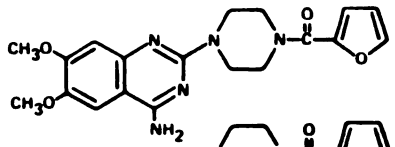
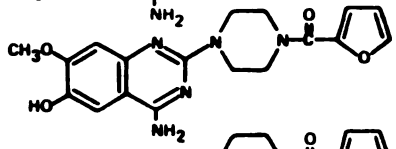
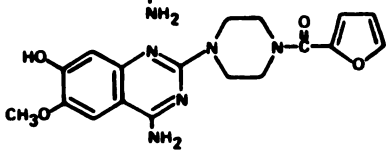
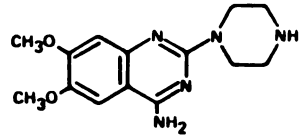
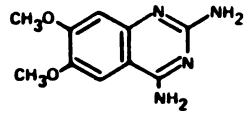
Product	Structure	Excreted form	Per cent relative excretion							
			Dog				Rat			
			Urine	Bile	Total		Urine	Bile	Total	
Prazosin		Free Conjugated	5 ND	≤1 ND	6 ND	6	5 ND	≤1 ND	6 ND	6
6-*O*-Demethyl Prazosin		Free Conjugated	1 ND	15 50	16 50	66	1 ND	10 50	11 50	61
7-*O*-Demethyl Prazosin		Free Conjugated	≤1 ND	5 15	6 15	21	1 ND	2 10	3 10	13
2-(1-Piperazinyl)-4-amino-6,7-dimethoxyquinazoline		Free Conjugated	1 ND	≤1 ND	2 ND	2	5 ND	5 ND	10 ND	10
2,4-Diamino-6,7-dimethoxyquinazoline		Free Conjugated	1 ND	≤1 ND	2 ND	2	ND ND	≤1 ND	≤1 ND	2

Figure II-3. Percent relative excretion of prazosin in urine and bile from rats and dogs. Values represent percent of the recovered dose in urine and bile expressed as relative excretion. ND - product not detectable (<1%). [Reproduced from Taylor et al. (5)]

drug-related products was similar. This information has been referred to by others (38,114) as introductory studies indicating a pattern of hepatic metabolic degradation in man that is similar to that seen in dogs. Although this may be the case, data confirming this hypothesis has not been presented.

The activity in dogs of the reported metabolites was compared to prazosin at three different dosages (4) (see Table II-5). At a dose of 1 mg/kg, the 6- and 7-demethyl and 2,4-diamino compounds appear to have a demonstrated maximum relative hypotensive activity of 49, 44 and 60%, respectively. When the reduction in blood pressure and the dose of each compound required to produced that response is compared, a 10-fold difference in activity would be apparent. Due to an insufficient number of doses of each compound used in this experiment, a clear appraisal of relative activity cannot be elucidated from this data.

In a separate study (113), pooled plasma samples from several human subjects were analyzed by TLC and a single material, with the R_f of prazosin, was observed. When blank plasma from the same human subject was spiked with three dog metabolites and analyzed by TLC, "several zones were evident under the same conditions" (113). The authors suggest from this data that prazosin metabolites are not present in the plasma of human subjects.

Table II-5. Hypotensive activity of prazosin and metabolites in dogs. [From Althius and Hess (4)]

Compound	Maximum decrease in blood pressure (mm) observed with increasing doses (iv) administered at 30 min intervals[a]		
	0.1 mg/kg	0.4 mg/kg	1.0 mg/kg
prazosin (XI) hydrochloride	20[b]	45[b]	55[b]
6-O-demethylprazosin (XII) sulfate	NT[c]	10 - 17	15 - 27
7-O-demethylprazosin (XIV) sulfate	NT[c]	12 - 15	18 - 24
2,4-diamino-6,7-dimethoxyquinazoline (XVII) free base	0	3 - 10	23 - 33
2-(1-piperazinyl)-4-amino-6,7-dimethoxy-quinazoline (XVI) hydrochloride	0	0	10 - 16

[a]Values given are for the amine salt indicated and are uncorrected for differences in molecular weight; the average control value was 130 mm Hg.

[b]Mean value from 6 dogs; values for metabolites are ranges obtained with the dogs tested.

[c]Not tested

In 1980, Rubin and Blaschke (108) reported a poor correlation between prazosin blood concentrations and the pharmacologic effect (to be discussed in Section II.E - Dose Response). They observed that the peak effect on blood pressure occurred after the peak plasma concentration, and suggested that metabolites may play a role in the action of prazosin. Bateman and Rawlins (136) questioned this hypothesis, but the possible importance of pharmacologically active metabolites was later reiterated in a discussion by Pitterman et al. (121). These investigators found a prolonged effect following oral prazosin administration and discussed, at length, the probability of a higher fraction of active metabolites being formed after oral administration than following iv dosing.

The high concentrations of metabolites (drug-related materials) in the bile of rats and dogs clearly suggests that the metabolism of prazosin takes place in the liver. Urine collection of adequate duration from normal subjects (117) and hypertensives with normal renal function (123,124) have, in each case, shown that less than 4% of the parent compound is excreted unchanged in the urine. From this data we would suspect that any disease entity affecting liver function would contribute to alterations in the metabolic profile of prazosin.

It is quite apparent that the knowledge concerning the metabolism of prazosin is incomplete. Further studies,

specifically the isolation and identification of metabolites, and the effect of disease states on prazosin metabolism, are required.

D. Elimination

In two studies plasma clearance (CL_p) following a 1 mg intravenous dose to young, normal volunteers has been reported, i.e., 12.7 ± 1.3 (117) and 18.3 ± 3.4 L/hr (120). In elderly subjects the plasma clearance, 16.5 ± 4.7 L/hr (120), was not significantly different. Flouvat et al. (123) and Grahnen and coworkers (124) reported CL_p values in hypertensive patients with normal renal function to be 9.8 ± 2.6 and 12.1 ± 4.2 L/hr, respectively.

The reported half-lives of prazosin in normal, hypertensive (with and without chronic renal failure) and congestive heart failure patients are presented in Table II-6. Individuals with hypertension, regardless of renal status, appear to have the same elimination half-life as do normal controls. Individuals with chronic congestive heart failure have a prolonged half-life, apparently related to a decreased metabolic ability in this patient group. Bateman et al. (117) estimated an hepatic extraction ratio (E) of 27%, and Grahnen and coworkers (124) estimated E to be 14% in their study of hypertensive patients. This would imply that prazosin is a low extraction ratio compound, and as

Table II-6. The half-life of prazosin as reported in normal, hypertensive and congestive heart failure patients. (Mean ± SD)

Author/year ref	Type of subject	# of subjects	Dose (mg)	Dosage form	Half-life (hrs)	Range (hrs)
Wood et al.,1976 (112)	normal	10	5	tab	3.8 ± 0.8	NR
Hobbs et al.,1978 (113)	normal	24	5	cap	2.7 ± 0.7	1.8 - 4.6
	normal	24	5[a]	cap	2.6 ± 0.8	1.7 - 5.2
	normal	21	5	soln	2.3 ± 0.3	1.7 - 2.8
Bateman et al.,1979 (117)	normal	6	1	tab	2.9 ± 0.6	2.2 - 3.7
	normal	6	1	iv	2.6 ± 0.5	2.0 - 3.5
Dynon et al.,1980 (119)	normal	5	0.5[b]	tab	2.7 ± 1.2	1.4 - 4.3
	normal	5	1.5	tab	3.1 ± 1.6	1.5 - 5.2
Pitterman et al.,1981 (121)	normal	5	5	cap	2.9 ± 0.7	NR
Rubin et al.,1981 (120)	normal[c]	7	1	oral	2.0 ± 0.3	NR
			1	iv	2.1	NR
	normal[d]	7	1	oral	3.1 ± 0.6	NR
			0.5	iv	3.2	NR
Jaillon et al.,1979 (118)	normal	10	5	cap	2.4 ± 0.2	NR
Collins & Pek,1975 (122)	HTN[e]	4	2-5	oral	3.6	1.5 - 5
Simpson et al.,1977 (116)	HTN	4	chronic	oral	6 - 8	NR

Table II-6. (continued)

Author/year ref	Type of subject	# of subjects	Dose (mg)	Dosage Form	Half-life (hrs)	Range (hrs)
Flouvat et al.,1979 (123)	HTN	10	2[f]	tab	3.1 ± 1.1	1.7 - 5.0
		10	2	iv	2.9 ± 0.9	1.7 - 5.0
Lowenthal et al.,1980 (126)	HTN/NRF[g]	NR	1-2	oral	3.7 ± 2.2[i]	NR
	HTN/CRF[h]	NR	1-2	oral	4.8 ± 0.9[i]	NR
Grahnen et al.,1980 (124)	HTN	8	0.5[b]	tab	3.0 ± 0.6	2-4
	HTN	4	0.5	iv	2.8 ± 0.6	2.3 - 3.3
Chaignon et al.,1981 (125)	HTN/NRF	9	2[f]	tab	3.6 ± 1.2	2.0 - 5.2
	HTN/CRF	9	2[f]	tab	2.9 ± 0.9	1.7 - 4.7
Jaillon et al.,1979 (118)	CHF[j]	9	2	oral	6.2 ± 1.7	NR
Silke et al.,1981 (127)	CHF	8	0.5	oral	5.5	NR
		8[k]	1	oral	5.3	NR
	CHF	5[k]	2	oral	5.6	NR
		5[k]	4	oral	5.9	NR

[a] two 2 mg and one 1 mg capsules

[b] one-half of a 1 mg tablet

[c] young normal (22-32 years)

[d] elderly normal (66-78 years)

[e] Hypertension

[f] two 1 mg tablets

[g] Normal Renal Function

[h] Chronic Renal Failure

[i] Mean ± SEM

[j] Congestive Heart Failure

[k] The same patients were used throughout this study, but only 5 could tolerate oral doses greater than 2 mg.

such its clearance will depend on the ability of the liver to metabolize the compound, and not on the rate of blood flow to the clearing organ (the liver).

E. Dose-Response Relationship

In ten normotensive individuals who received single 5 mg doses of prazosin, Wood et al. (112) observed that the effect of prazosin on blood pressure did not closely correlate with the plasma concentration following oral administration of the drug. The authors reported that in spite of a mean terminal half-life of 3.8 hours, satisfactory hypertension control was evident when the drug was dosed only twice daily. The authors suggested that tissue concentrations of prazosin at the site of action follow a much different time course than the plasma concentrations.

A statistically significant correlation between log-plasma prazosin concentration and the change in both orthostatic systolic and diastolic pressures following intravenous administration were reported by Bateman and coworkers (136). They also found a significant correlation after iv dosing between log-plasma concentration of the drug and the postural fall in systolic and diastolic blood pressure. As in previous work (112) no correlation between the plasma concentration and the cardiovascular actions of prazosin was found after oral doses. The correlation between the plasma

prazosin concentration and the effects on supine and orthostatic blood pressure and heart rate in eight patients with severe refractory heart failure was examined by Silke and coworkers (127). A clear dose-response effect on blood pressure and heart rate was evident only when the patients were standing. This finding was supported further by the work of Larochelle et al. (137).

Lowenthal et al. (126) and Chaignon et al. (125) studying hypertensive subjects reconfirmed the extended duration of activity versus the peak plasma concentration, further indicating a "deep" effect compartment. These authors also reported that hypertensive patients with chronic renal failure receiving daily low doses of prazosin have smaller AUC, but demonstrate hypotensive response similar to hypertensive patients with normal renal function. It was proposed (126) that this similar effect at lower concentrations could be due to a greater sensitivity to prazosin in patients with abnormal renal function. The poor correlation between the blood (or plasma) concentration and the pharmacologic effect may well reflect the complex nature of blood pressure control (86). The suggestion that active metabolites may be contributing to the overall pharmacologic effect was made by both Rubin and Blaschke (107) and Pitterman and coworkers (121).

Seideman et al. (138) studied pharmacological effects in relation to the kinetics of prazosin after single iv and

oral doses (0.5 mg), and during multiple oral doses. They confirmed that prazosin concentrations correlated with the dose, and that following iv administration the fall in systolic and diastolic blood pressure correlated with the prazosin concentration during the terminal phase in all patients studied. In five of eight patients taking continuous prazosin therapy with increasing doses, there was a significant correlation ($p<0.05$) between steady-state plasma concentrations and the decrease in blood pressure, but in the group as a whole there was no correlation. These authors, as well as Jaillon (114), see little clinical use for blood (or plasma) concentration monitoring of prazosin.

F. Drug Interactions

Two types of interactions may alter the effect of prazosin: 1) agents that reduce or negate the reduction in blood pressure, and 2) other pharmacological compounds that, when coadministered, alter the pharmacokinetic profile of the compound. Rubin et al. (139) studied both types of interactions, assuming that a concentration-effect relationship does exist for the parent drug and metabolites. It had been suggested that indomethacin, or a similar compound, may, by its sodium and water retaining effects, attenuate prazosin-induced hypotension. After dosing nine subjects to steady-state with indomethacin, prazosin was dosed con-

currently, and hemodynamic studies were performed. In four of the nine normal subjects, indomethacin reduced the fall in blood pressure secondary to prazosin, while in the remaining subjects there was no change. Although the mechanism for this effect was not elucidated, the authors suggested that in some individuals indomethacin may alter adrenergic sensitivity.

The beta-blocker propranolol has been shown to decrease the clearance of high hepatic extraction compounds (those agents showing hepatic blood flow limited clearance) (140). Following seven days pretreatment with propranolol, five subjects took 80 mg of propranolol and 5 mg prazosin, both orally. The pharmacokinetics of prazosin and hemodynamic changes were assessed over eight hours. No effect of propranolol on prazosin disposition or on prazosin-induced hemodynamics was observed, which is in agreement with the low extraction ratio reported by Grahnen et al. (124).

CHAPTER III

PRAZOSIN ANALYTICAL METHODOLOGY

A. Previous Assay Methods

Several methods for the measurement of prazosin have previously been reported. These methods are similar in that they are high performance liquid chromatographic (HPLC) procedures that quantitate the compound by fluorescence detection. Common to all of these procedures is extraction of the basic sample into ethyl acetate. Verbesselt et al. (115), in 1976, briefly described the extraction procedure for their analytical method. Their sample preparation involved adding base (1.0 ml 2N NaOH) to the plasma sample, extracting into 15 ml ethyl acetate, acidifying the organic layer with 2.5 ml 0.1N H_2SO_4 and then assaying the aqueous fraction. This method requires 4 ml of plasma and is sensitive to 1 ng/ml. Simpson et al. (116) detailed a similar extraction method sensitive to 2 ng/ml, which was quite similar to that used by Verbesselt et al. (115). All of these assay methods require a large plasma sample (3 ml) and are cumbersome to perform due to quantitative or carefully controlled transfer steps. In 1978 an improved assay from Pfizer Central Research was published by Twomey and Hobbs (141). The need for the quantitative transfer steps had been eliminated by incorporation of an internal standard.

Unfortunately, two extraction steps are necessary and the internal standard is not readily available.

The quantitative transfer procedure of the earlier methods and the double extraction required in the Pfizer assay make the analysis of a large number of samples a lengthy and involved process. Thus, to decrease the sample preparation time and increase the number of samples that can be analyzed in one day new methods for the determination of prazosin in human plasma, whole blood and urine were developed. The methods are simple, rapid, sensitive and involve no extraction steps and require only 0.2 ml of biological sample.

B. Experimental

Two different HPLC analytical methods that measure prazosin by fluorescence detection were developed. Method I incorporates the use of carbamazepine (XVIII, see Figure III-1), quantitated by UV detection, as the internal standard (IS). Method II uses tiodazosin (see Figure III-2), a new aminoquinazoline compound, as the internal standard and quantitates both compounds utilizing fluorescence detection only. Method I allows for the adjustment of the sensitivity on one detector (or recorder) without an obligatory alteration in the sensitivity of the other. If both compounds are analyzed with same detector, a prior estimation of the pra-

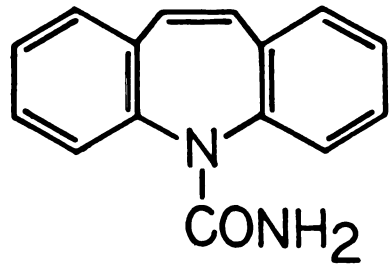
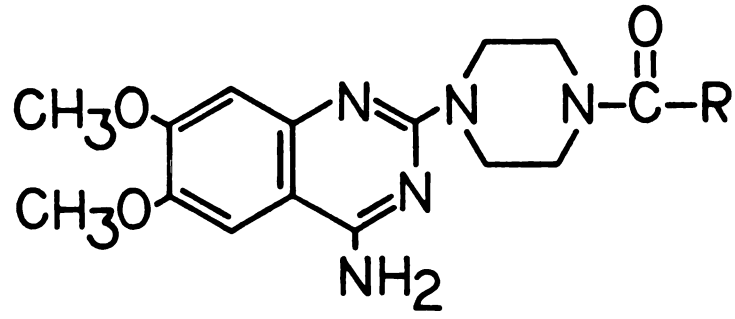
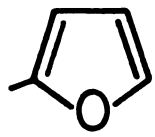


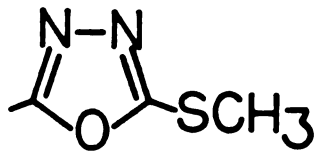
Figure III-1. Structure of carbamazepine (XVIII), internal standard for prazosin assay - Method I.



R



PRAZOSIN



TIODAZOSIN

Figure III-2. Structural comparison of prazosin and tiodazosin.

zosin concentration range is required so that an appropriate IS concentration can be selected. Method I would be chosen for analysis of small volume samples because it is not necessary to be concerned about the assay sensitivity range prior to analysis, and thus reduce the volume of sample ultimately required for assay. Method II uses an internal standard structurally similar to prazosin that possesses similar fluoresecent properties. In this way only fluorescence detection is required and, although knowledge of the relative concentration range is necessary so that prazosin at both ends of the concentration range and the IS can be detected with good sensitivity, one compound can serve as the internal standard for the other.

1. Reagents

Prazosin hydrochloride[1], supplied as prazosin standard 7866-271-A, tiodazosin levulinate[2] (BL5111R) tiodazosin standard 77F655, and carbamazepine[3] (Tegretol^{R}) were used as received from the suppliers. Glacial acetic acid[4] and phosphoric acid[5] were analytical reagent grade and the methanol[6]

---

[1]Pfizer Inc., Groton, CT

[2]Bristol Laboratories, Syracuse, NY

[3]Geigy Pharmaceuticals, Division of Ciba-Geigy Corp., Ardsley, NY

[4]Mallinckrodt Chemical, St. Louis, MO

[5]Fisher Scientific, Fair Lawn, NJ

[6]J.T. Baker Chemical Co., Phillipsburg, NJ

and acetonitrile[6] were HPLC grade. Steam distilled water was glass re-distilled and then deionized and filtered through a 120-volt NANOpure[R] system[7].

2. Instrumentation

Method I

A Perkin-Elmer[8] (PE) Series 2 Liquid Chromatograph equipped with a rotary valve injector[9], a PE Fluorescence Spectrophotometer (Model 204A or Model 650-10S), a PE fixed wavelength (254 nm) UV detector (Model 250) and a Linear[10] Model 300 Series dual pen recorder were used with a C-18 μBondapak[R] reversed phase column[11] (3.9 mm ID X 30 cm, 10 μ particle size). The fluorescence detector was operated at an excitation wavelength of 340 nm and an emission wavelength of 384 nm. Due to the narrow bandwidth (5 nm) of the fluorometer, only prazosin is detectable under the conditions described.

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[7]Barnstead Co., Division of Sybron Corp., Boston, MA
[8]Perkin-Elmer Corp., Instrument Division, Norwalk, CT
[9]Rheodyne Inc., Cotati, CA
[10]Linear Instrument Corp., Irvine, CA
[11]Waters Associates, Milford, MA

Method II

A Varian[12] Model 5000 Liquid Chromatograph equipped with a Waters[11] Intelligent Sample Processor, a PE fluorescence spectrophotometer (Model 650-10S) and a Curken[13] dual-pen recorder were used with a C-18 reversed phase column[14] (25 cm X 4.6 mm ID, 10 μ particle size). As in Method I, the fluorescence detector was operated at an excitation wavelength of 340 nm and an emission wavelength of 384 nm.

3. Preparation of stock solutions

Prazosin standard (approximately 2.5 mg) was placed in 5 ml 95% ethanol, vortexed and then sonicated for 3 minutes to insure complete solubility. This solution was then diluted with distilled water to 50 ml in a graduated volumetric flask. The prazosin solutions used to spike plasma or whole blood samples for individual standard curves were prepared by diluting aliquots of the stock solution 1:500 with distilled water.

Carbamazepine, typically 10 mg, was dissolved in 50 ml methanol (HPLC grade). This solution was found to be

[12]Varian Associates, Palo Alto, CA
[13]Curken Scientific, Danbury, CT
[14]Alltech Associates, Deerfield, IL

unstable after two weeks, even when refrigerated, so fresh stock carbamazepine solutions were prepared every third day. For addition to samples, a 300 µl aliquot of the stock solution was diluted to 40 ml with acetonitrile (1:133).

Tiodazosin (approximately 3.0 mg) was dissolved in 5 ml of ethanol (95%) and diluted to 50 ml with distilled water. For the preparation of standard curves, 10 µl of tiodazosin stock solution was added to 5 ml distilled water (1:500 dilution) and the resulting solution used to fortify the blank biological samples in varying concentrations.

4. Assay of prazosin in plasma and whole blood

All samples (0.2 ml plasma and whole blood) were deproteinated by adding 0.4 ml of CH_3CN containing the internal standard. After vortexing for 30 seconds and centrifuging for ten minutes at 3200 rpm (1500 X g) in an IEC[15] HN-S centrifuge, the supernatant was transferred to a clean test tube and evaporated to approximately 100 µl under nitrogen gas. An appropriate aliquot of the remaining sample was then injected onto the column.

[15]International Equipment Co., Needham Heights, MA

Method I

The mobile phase was a solution of 43% (V/V) methanol in water with 0.6 ml glacial acetic acid, pH adjusted to 5.0 with sodium hydroxide solution, which was filtered (Whatman No. 2 filter paper) and degassed prior to use. The solvent pump was operated at a flow rate of 2.0 ml/min under isocratic and ambient temperature conditions.

Method II

The mobile phase was a solution of 21% (V/V) acetonitrile in water with 0.1% (V/V) phosphoric acid. The pH of the mobile phase was adjusted to 3.6 with a sodium hydroxide solution and, as in Method I, was filtered and degassed prior to use. The solvent was pumped isocratically at a flow rate of 2.0 ml/min at ambient temperature.

5. Assay of prazosin in urine

A 400 µl aliquot containing the internal standard, carbamazepine, was added to each 0.2 ml prazosin urine sample. The solution was mixed well for 30 seconds on a vortex mixer and then evaporated to about 100 µl with a gentle stream of nitrogen gas. A sample, typically 50 µl, was injected onto the column. Instrument settings and mobile phase

composition were identical to those for plasma analysis by Method I.

6. Assay of tiodazosin in plasma and whole blood

The sample preparation for tiodazosin analysis is the same as the assay for prazosin in plasma, except that prazosin (approximately 180 ng/ml) was used as the internal standard. Under these conditions and following evaporation by N_2, 15 to 30 µl was injected onto the column. Mobile phase composition was identical to that of Method II.

C. Results and Discussion

1. Prazosin assays

Representative chromatograms (Method I) of spiked plasma used for calibration curves and plasma from a human subject, before and following prazosin oral administration, are shown in Figure III-3. Control samples of plasma, whole blood and urine show no interfering peaks. The retention times for prazosin and carbamazepine are 7 and 10 minutes respectively. The limit of quantitation (signal-to-noise ratio 3:1) for prazosin with this method is 0.1 ng/ml in plasma, whole blood and urine.

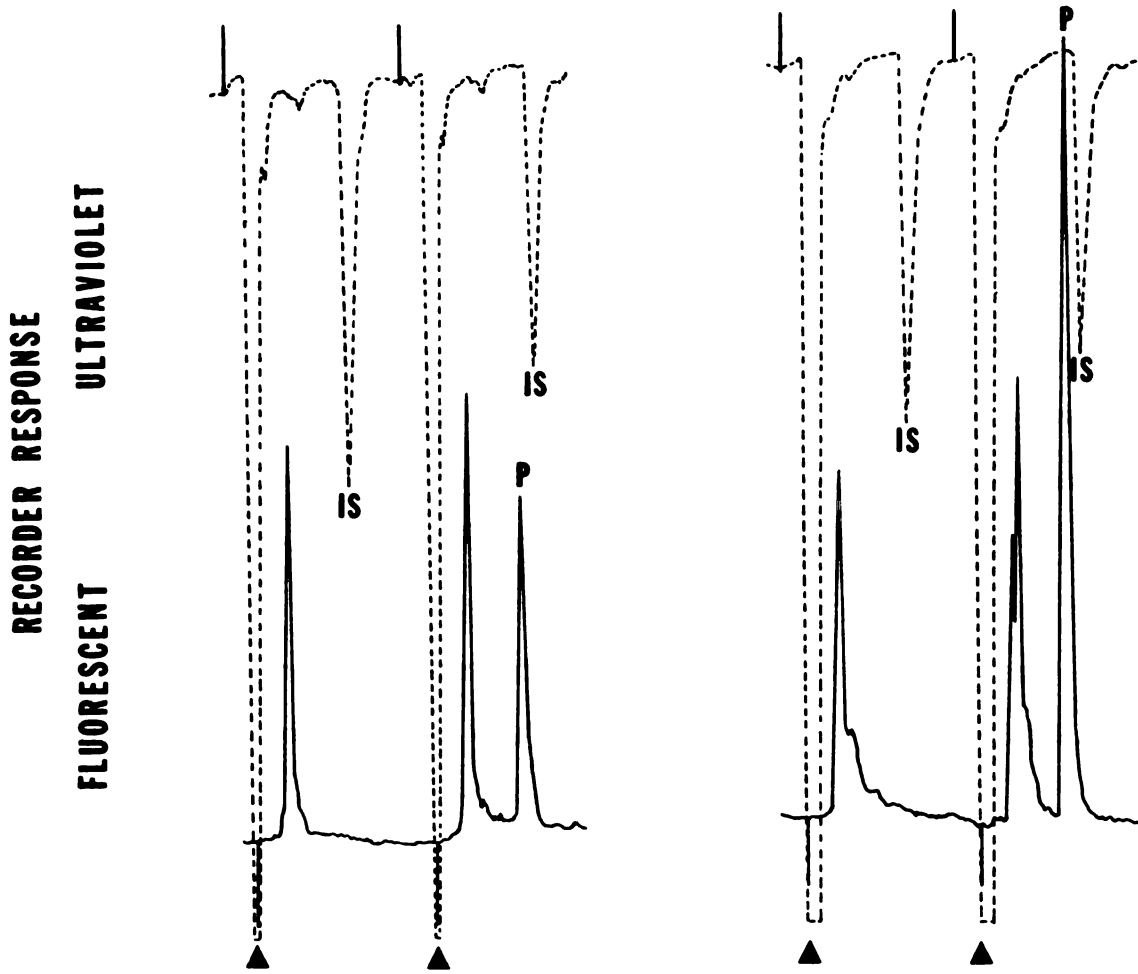


Figure III-3. HPLC chromatograms of human plasma when assayed for prazosin using Method I.

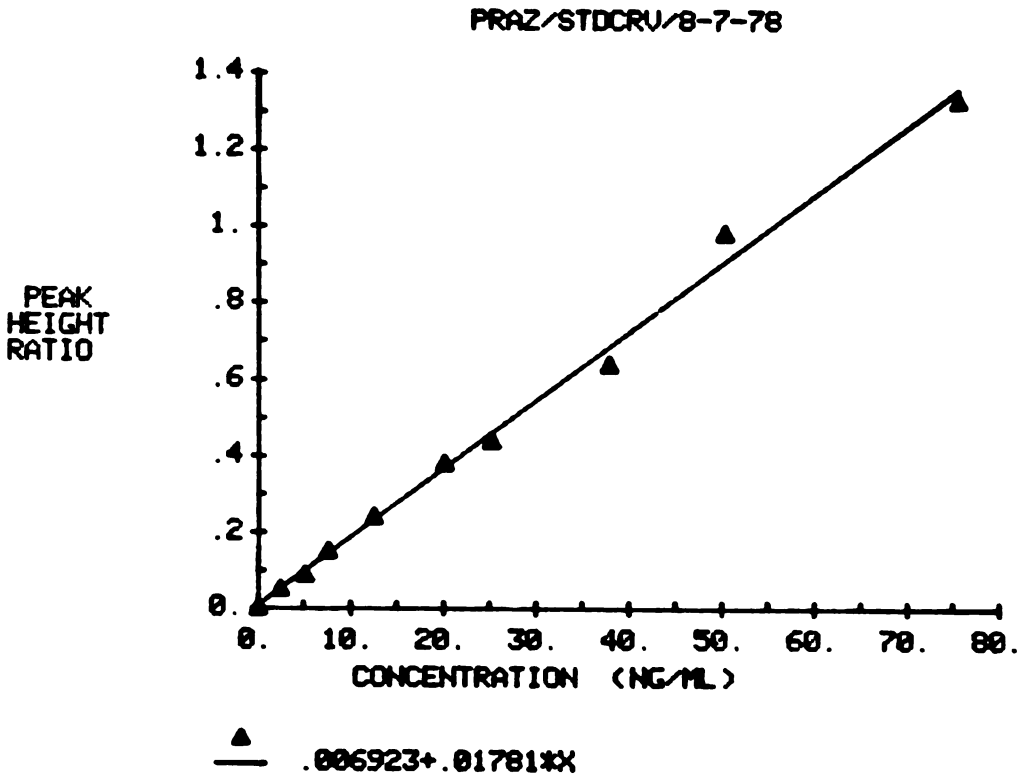
LEFT: Dual-pen recording of chromatogram for blank plasma and plasma spiked with prazosin. In the dual-pen chromatograms the UV response is shifted to the left of the fluorescent response, such that the marked solid line on the UV tracing corresponds to the inject symbol on the fluorescent response. Symbols (retention times in parentheses): ▲, inject; P, prazosin (7 min); IS, internal standard (10 min).

RIGHT: Dual-pen recording of chromatograms for blank plasma and plasma sample from a normal subject. Blank sample and sample obtained 2 hours after a 5 mg oral dose of prazosin.

Calibration graphs were constructed from spiked plasma and urine samples using the sample procedure described in Section III.B.4. Prazosin was added to provide a standard curve concentration range of 2 - 76 ng/ml in actual studies, although calibration curves were shown to be linear from 1 - 164 ng/ml. The peak height ratios (prazosin/IS) were plotted versus drug concentration (in ng/ml), and the calibration graph was used for the calculation of the plasma, whole blood or urine concentrations in human subjects (see Figure III-4). All calibration graphs were linear over the concentration ranges measured and a mean coefficient of determination for eleven calibration curves was 0.993, with a coefficient of variation of 0.39%.

A representative chromatogram of human urine before and after prazosin administration is seen in Figure III-5. Prazosin concentrations in the urine were found to be the same when determined from calibration curves constructed in either urine or plasma.

Figure III-6 is a chromatogram for the analysis of prazosin in plasma using Method II. Under these chromatographic conditions, prazosin and tiodazosin have retention times of 8 and 12 minutes, respectively. Calibration curves were constructed over two concentration ranges, depending on the dosage of prazosin administered. The first calibration graph typically includes samples from 0.9 to 46 ng/ml, which results in the following linear least squares regression



NUMBER OF DATA POINTS = 10
CORRELATION COEFFICIENT R = .9974835 R-SQUARED = .9949734
STANDARD DEVIATION OF REGRESSION = .03286084

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	.006923016	.01486666	.4656738	.654
SLOPE	.01781377	.0004476528	39.79371	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	1.709961	1.	1.709961	1583.539	.0001
RESIDUAL	.00863868	8.	.001079835		

Figure III-4. Prazosin standard curve in human plasma with statistical evaluation.

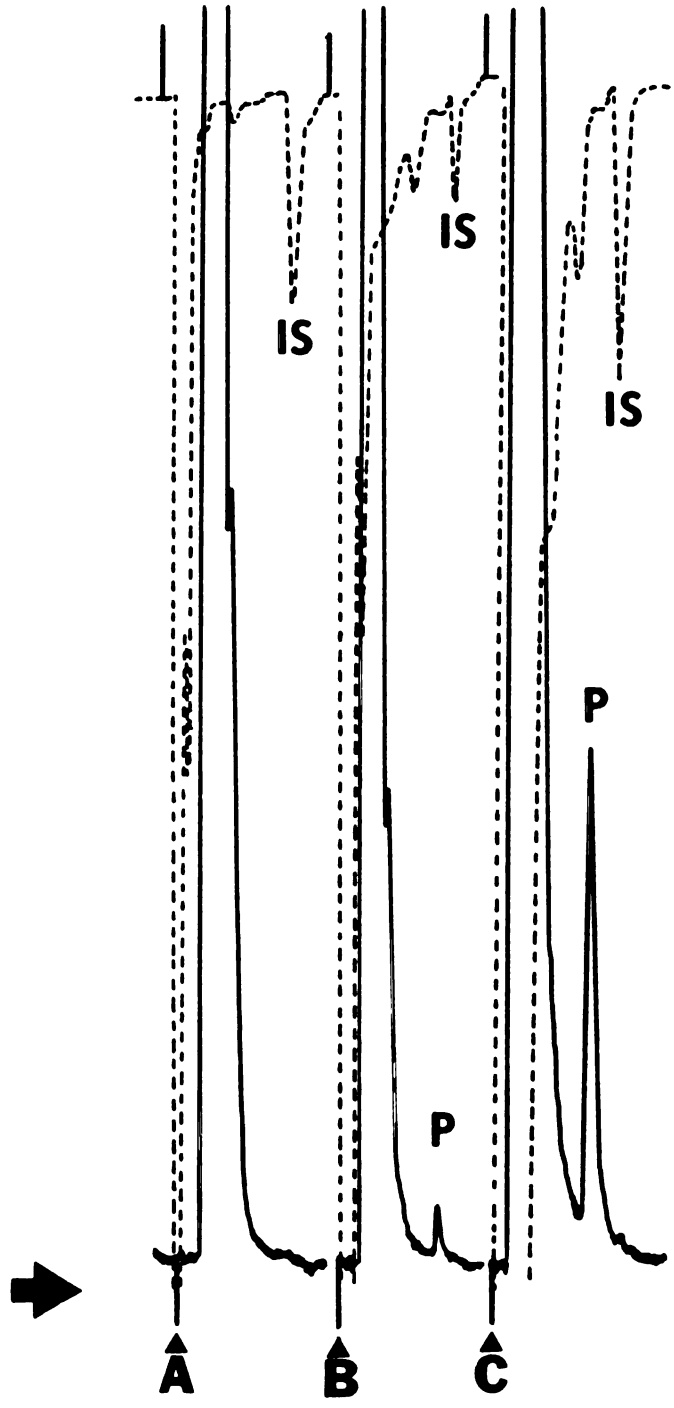


Figure III-5. HPLC chromatograms of human urine before and after prazosin administration. A) blank urine; B) urine collected over first 24 hours after prazosin dosing (25.3 ng/ml); and, C) urine collected from 24-48 hours following prazosin administration (91.9 ng/ml). All samples were from the same subject.

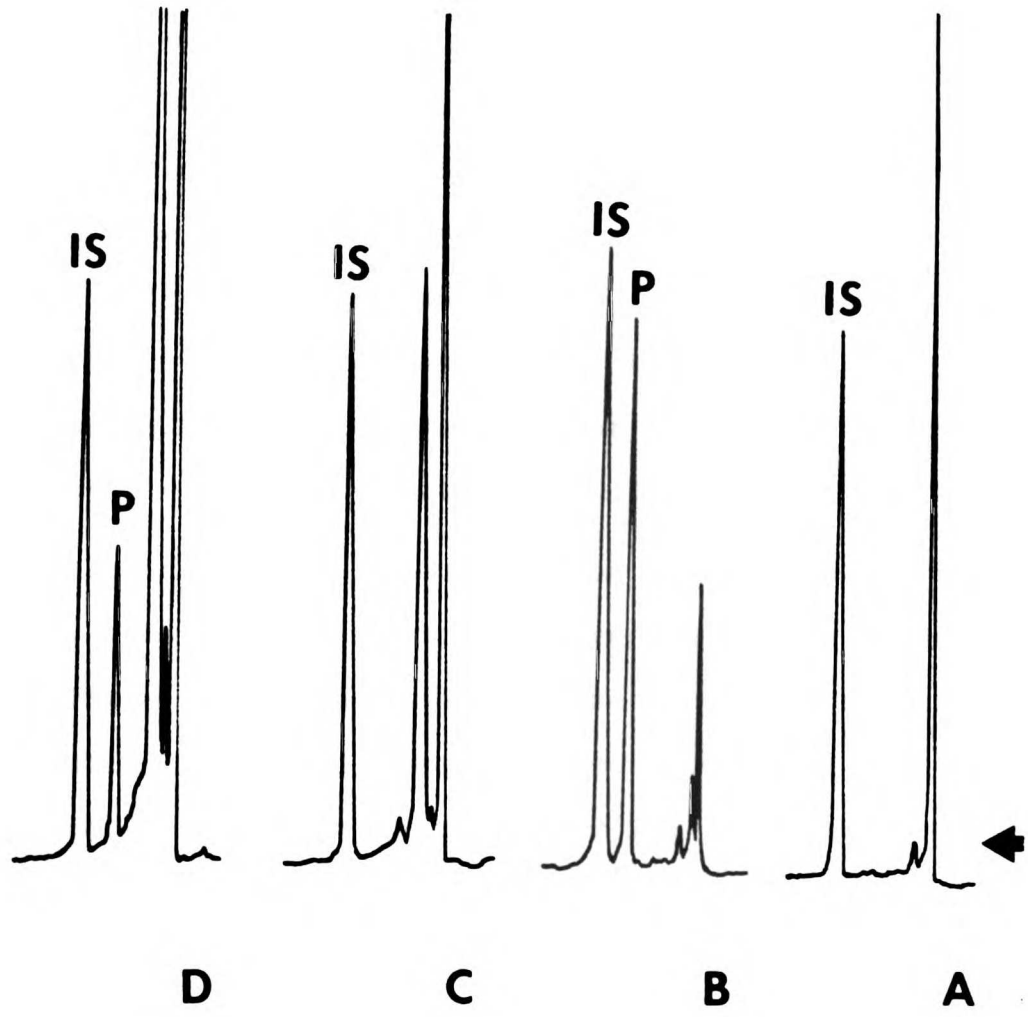
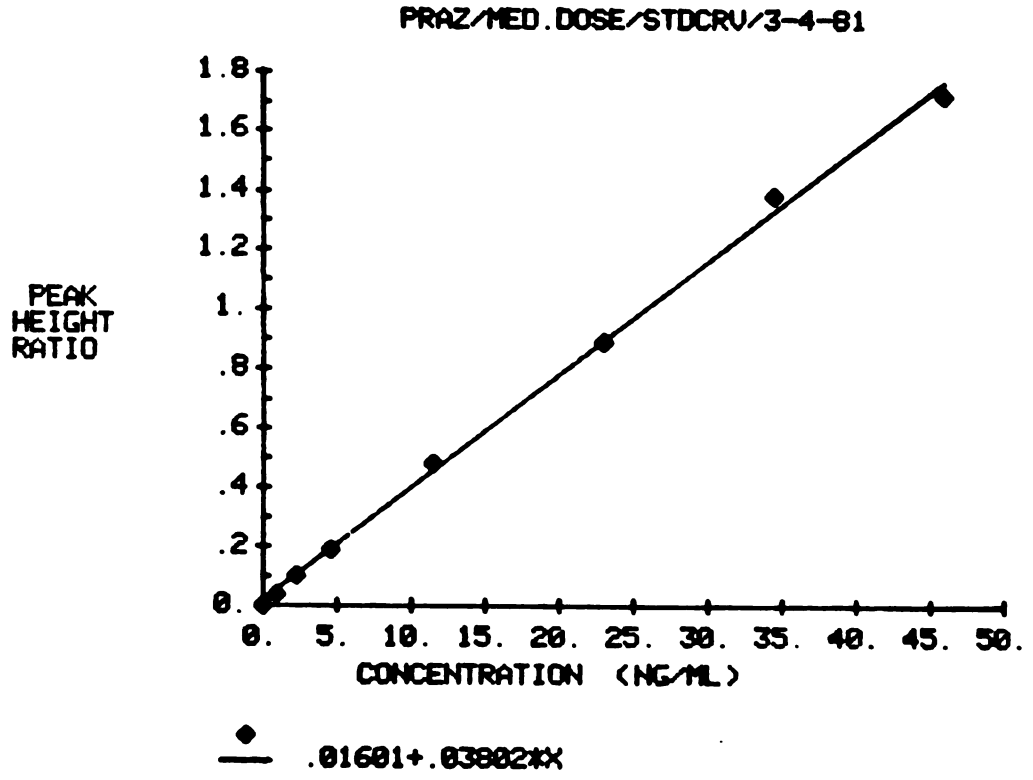


Figure III-6. HPLC chromatogram of dog plasma using Method II. A) standard curve blank; B) plasma spiked with prazosin to 23 ng/ml; C) blank plasma prior to oral dosing; and, D) following oral administration (14.2 ng/ml).

equation: $Y = 0.038X + 0.016$, $r^2 = 0.998$ (Figure III-7). This standard curve was used for the analysis of plasma and whole blood from dogs receiving 5 to 15 mg oral prazosin. The second calibration curve (labelled "lo-dose standard curve" so as not to be confused with other prazosin calibration curves) is constructed over the concentration range of 0.23 - 11.5 ng/ml. This "lo-dose" standard curve was used for whole blood and plasma analysis from dogs that received oral prazosin doses less than 5 mg. A representative linear least squares regression equation for the "lo-dose standard curve" is: $Y = .277X - 0.044$, $r^2 = 0.997$ (see Figure III-8).

The acetonitrile precipitation method was used to determine the extent of prazosin recovery from plasma proteins. Three sets of six samples (sets designated A through C in Table III-1), with each set consisting of two samples containing 0.2 ml water (Water-1 and 2), and four samples containing 0.2 ml plasma (Plasma-1 to 4) were prepared at three different concentrations (17, 54 and 118 ng/ml) (Method I). The absolute peak heights of prazosin in water and plasma were compared after injecting identical volumes (50 µl) of supernanant obtained following sample preparation (described in section III.B.5). The results (Table III-1) show that virtually all of the compound is removed from protein, that is, no difference is observed between the water and plasma samples.



NUMBER OF DATA POINTS = 8
CORRELATION COEFFICIENT R = .9990327 R-SQUARED = .9980663
STANDARD DEVIATION OF REGRESSION = .03137959

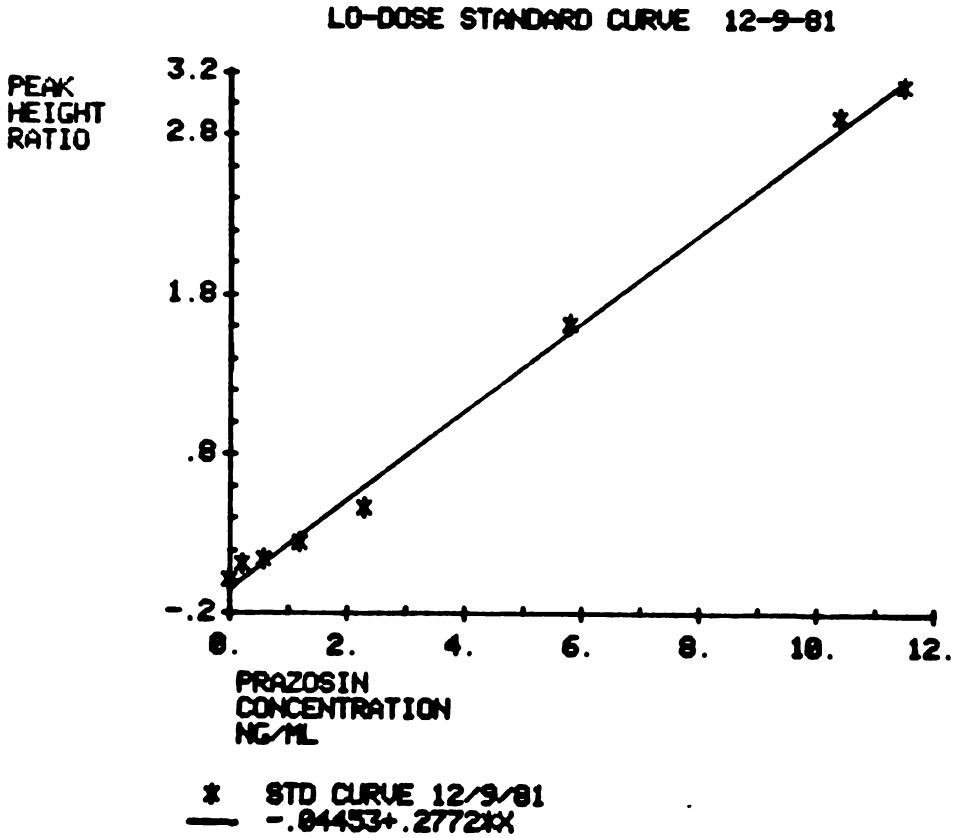
PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	.0160058	.01526829	1.048303	.335
SLOPE	.03802275	.0006832634	55.64874	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	3.049335	1.	3.049335	3096.782	.0001
RESIDUAL	.005908072	6.	.0009846787		

Figure III-7. Prazosin standard curve constructed with dog plasma over the "medium" concentration range with statistical analysis.



NUMBER OF DATA POINTS = 8
CORRELATION COEFFICIENT R = .9983442 R-SQUARED = .9966911
STANDARD DEVIATION OF REGRESSION = .00066868

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	-.04452954	.03865196	-1.152064	.2931
SLOPE	.2771708	.006519774	42.51233	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	11.76088	1.	11.76088	1807.298	.0001
RESIDUAL	.03904462	6.	.006507436		

Figure III-8. Prazosin "lo-dose" standard curve constructed in dog plasma with statistical analysis.

Table III-1. Recovery of prazosin from plasma proteins.

Set/ Concentration	Sample	prazosin peak height (cm)	carbamazepine peak height (cm)	Peak Height Ratio
A	Water-1	3.7	10.5	.35
	Water-2	3.7	10.45	.35
17.0 ng/ml	Plasma-1	3.9	10.8	.36
	Plasma-2	3.9	10.65	.36
	Plasma-3	3.6	10.5	.34
	Plasma-4	3.7	11.2	.33
B	Water-1	11.0	10.9	1.01
	Water-2	10.9	10.3	1.06
54.0 ng/ml	Plasma-1	10.6	10.8	0.98
	Plasma-2	10.9	10.8	1.01
	Plasma-3	11.0	10.8	1.01
	Plasma-4	10.8	10.2	1.06
C	Water-1	24.0	10.9	2.20
	Water-2	24.0	9.7	2.47
118 ng/ml	Plasma-1	24.8	10.6	2.32
	Plasma-2	23.8	10.1	2.36
	Plasma-3	23.6	10.8	2.18
	Plasma-4	25.0	10.6	2.36

The within-day precision of this method was assessed by conducting replicate analysis (n=10) of the same fortified plasma sample. Coefficients of variation of 1.35, 1.85, 8.50 and 0.82 % were determined for samples with mean plasma concentrations of 6.5, 13.0, 19.5 and 46.0 ng/ml, respectively.

Frozen plasma samples of various concentrations were found to be stable through the three weeks tested (see Table III-2).

2. Tiodazosin assays

Representative chromatographs of plasma spiked with tiodazosin and plasma from a beagle dog after prazosin administration are shown in Figure III-9. The chromatographic conditions of Method II yield baseline separation of tiodazosin from the internal standard, prazosin. As with prazosin assays utilizing Method II, retention times for tiodazosin and the IS are 8 and 12 minutes, respectively. As shown in Figure III-9, the use of the narrow bandwidth spectrofluorometer allows detection of tiodazosin and internal standard in plasma and whole blood without interfering peaks even though no extraction step is involved. HPLC chromatograms of whole blood samples (Figure III-9) were essentially identical to those of plasma samples. Analysis of urine samples from beagle dogs by Method II yielded

Table III-2. Effect of frozen storage on prazosin concentrations in plasma.

Prazosin[a] (ng/ml)	Time (days)						MEAN (ng/ml)	CV (%)
	0	1	2	3	14	21		
4.3	4.2	4.7	5.1	4.6	3.9	4.1	4.3	10.3
8.9	8.9	9.0	8.9	8.2	8.1	8.4	8.6	4.6
31.1	31.2	30.5	33.0	31.4	28.1	30.8	30.8	5.2

[a]spiked concentration

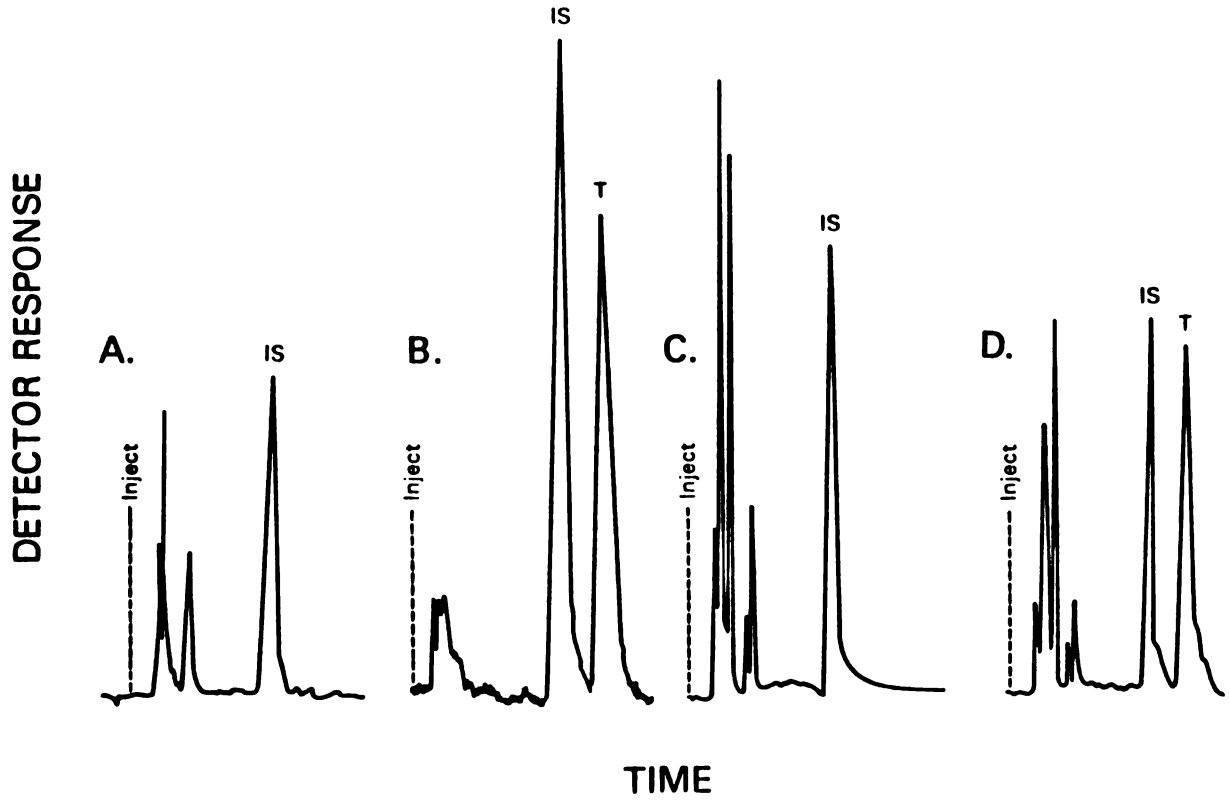
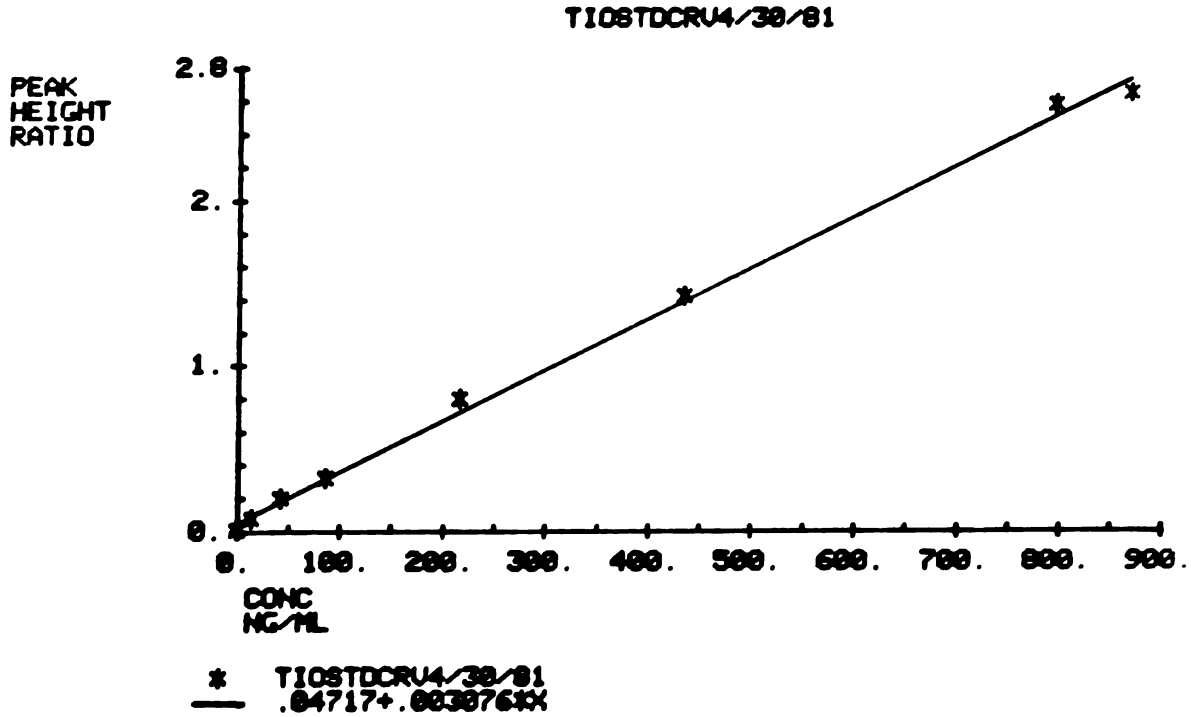


Figure III-9. HPLC fluorescence chromatograms for tiodazosin from blank plasma, blank whole blood, spiked plasma and spiked whole blood containing the internal standard, prazosin. Symbols (retention time in parentheses): T, tiodazosin (12 min); IS, internal standard (8 min). (A) Blank plasma with internal standard (184 ng/ml), (B) plasma spiked with tiodazosin (217 ng/ml) and internal standard, (C) blank whole blood with internal standard (184 ng/ml), (D) whole blood sample (90 min) following tiodazosin administration to a beagle dog, T (290 ng/ml).

chromatograms with large interfering peaks. However, when urine samples are prepared using the extraction methods previously reviewed, chromatograms without interfering peaks are obtained.

Tiodazosin was quantitated by comparison of the peak height ratio of drug to IS with the calibration curves. The slopes of calibration curves constructed with spiked plasma samples over the range of 6 - 868 ng/ml were linear and highly reproducible (see Figure III-10). The mean slope of six calibration curves was 0.003145 with a coefficient of variation of 7.6%. The mean correlation coefficient of six standard curves was 0.993 with a coefficient of variation of 0.70% (see Table III-3). The within-day variation was estimated by conducting replicate analyses (n=6) of spiked plasma samples. At 14.5, 43.5 and 217 ng/ml of tiodazosin, the coefficients of variation of tiodazosin content were 9.4, 4.2 and 4.9%, respectively.

The recovery of tiodazosin from plasma proteins was essentially quantitative. As in the prazosin assay, three sets of samples (sets designated A through C in Table III-4), with each set consisting of two samples of water (Water-1 and 2) and three samples containing 0.2 ml plasma (Plasma-1 through 3) were prepared at three different concentrations. The recovery of 15, 50 and 100 ng/ml was 104, 103 and 101%, respectively (see Table III-4). The concentration of tiodazosin in frozen plasma and whole blood sam-



NUMBER OF DATA POINTS = 8
CORRELATION COEFFICIENT R = .9987485 R-SQUARED = .9974965
STANDARD DEVIATION OF REGRESSION = .05888278

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	.04717082	.02841649	1.65998	.148
SLOPE	.003075639	$6.28792X10^{-5}$	48.91346	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	8.295326	1.	8.295326	2392.527	.0001
RESIDUAL	.02080309	6.	.003467182		

Figure III-10. Tiodazosin standard curve constructed with dog plasma using Method II with statistical analysis.

Table III-3. The slopes and coefficients of determination of six calibration graphs constructed with spiked tiodazosin plasma samples over the concentration range of 6 to 869 ng/ml.

slope	r^2
0.003156	0.9986
0.003323	0.9998
0.003406	0.9831
0.002732	0.9996
0.003187	0.9900
0.003074	0.9890
$\bar{X}$ 0.003145	0.9933
± SD 0.000239	0.0070

Table III-4. Recovery of tiodazosin from plasma proteins.

Set/ Concentration	Sample	Peak Height Ratio
A	Water-1	0.115
	Water-2	0.115
15.0 ng/ml	Plasma-1	0.121
	Plasma-2	0.111
	Plasma-3	0.127
B	Water-1	0.395
	Water-2	0.333
50.0 ng/ml	Plasma-1	0.380
	Plasma-2	0.365
	Plasma-3	0.383
C	Water-1	0.758
	Water-2	0.761
100 ng/ml	Plasma-1	0.847
	Plasma-2	0.733
	Plasma-3	0.774

ples did not change after 29 and 51 days of storage demonstrating the stability of tiodazosin in biological samples.

D. Summary

Two different high performance liquid chromatographic assays (Method I and Method II) have been developed. Both methods are easy to perform and samples can be prepared quickly as no derivatization or extraction procedures are required. Method I has the advantage of being able to immediately assess prazosin concentrations, as preparatory chromatography establishing the concentration range, is not required. Further, Method I is able to quantitate prazosin in human urine. Method II is used to provide two channel recording (one channel for high sensitivity, the other for moderate or low sensitivity) when the concentration range of the samples and the appropriate concentration range for the IS standard is established beforehand. Method II has been used to measure prazosin concentrations in dog plasma and whole blood to 200 pg/ml.

CHAPTER IV

THE PHARMACOKINETICS OF PRAZOSIN IN BEAGLE DOGS: APPARENT DOSE-DEPENDENT BIOAVAILABILITY

A. Objectives

As discussed in Chapter I, prazosin hydrochloride is a potent vasodilating agent that is commonly used in the treatment of hypertension and congestive heart failure. Experiments detailing the bioavailability and disposition of this compound in these patients, or in normals, have been limited owing to the lack of an intravenous dosage form. Studies in rats, cats and dogs, appraising the cardiovascular effects of prazosin (142-145) following intravenous administration, have indicated that this route of prazosin administration is tolerated well even at relatively large doses. Although pharmacokinetic studies in animals have not been reported, preliminary work (5) suggests that the metabolism of prazosin in dogs is similar to that in man. Thus, canines appear to be able to receive large intravenous doses; they possess a similar prazosin metabolic profile to that of humans, and they are thought to be an appropriate animal model for the investigation of prazosin pharmacokinetics.

The objective of this series of experiments is to determine the bioavailability and disposition of prazosin in male beagle dogs following intravenous and oral administration.

B. Single Oral and Intravenous Dose Study

1. Experimental

a. Specific objectives

To determine the bioavailability and clearance parameters of prazosin in beagle dogs following 1 mg/kg oral and intravenous doses.

b. Study design

Four male beagle dogs[1] from 2-3 years of age and 12.5 to 16.5 kg body weight were studied. Food was restricted for 8 hours prior to receiving the oral or intravenous dose, with water available ad libitum. On the morning of study day 1, each animal had both forelegs shaved and then was

[1]Marshall Research Animals, North Rose, NY
[2]Alice King Chatham Medical Arts, Los Angeles, CA

placed in an animal sling.[2] A Deseret[3] E-Z Set 21[R] infusion set (21G X 3/4" with 12" tubing) was placed in a superficial vein in the left foreleg and a Deseret E-Z Set-PRN[R] intermittent infusion set (21G X 3/4" with 3" catheter) was placed in a superficial vein in the right foreleg. Prazosin hydrochloride powder[4] (lot#: 7866-271-A) was dissolved in 2 ml 95% ethanol[5] and brought to a final volume of 50 ml with bacteriostatic water for injection.[6]

After blank blood and plasma samples were taken, the infusion set was connected to a compact infusion pump[7] and the prazosin solution infused over 10 minutes. The infusion line was then washed with an additional 6 ml of normal saline[8] and the infusion set removed from the animal. Venous blood was drawn from the right foreleg via the indwelling cannula, which was kept patent with heparinized[9] saline [8] (10 USP units/ml). Samples were taken at 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 1440, 2160 and 2880 minutes following the midpoint of the infusion period and transferred to Vacutainer[R] tubes[10] containing heparin.

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[3]Deseret Pharmaceutical Co., Sandy, Utah

[4]Pfizer Inc., Groton, CT

[5]Gold Shield Chemical Co., Hayward, CA

[6]Abbott Laboratories, North Chicago, IL

[7]Harvard Apparatus Co., Ealing Corp., South Natick, MA

[8]Bacteriostatic Sodium Chloride Injection, Elkins-Sinn Inc., Cherry Hill, NJ

[9]Liquemin[R] Sodium, Organon Inc., W. Orange, NJ

[10]Becton-Dickinson Division, Becton, Dickinson and Co., Rutherford, NJ

After a 14-day interval, prazosin was administered as commercially available 5 mg capsules[4] (lot #: 89045). Three dogs received 15 mg and the fourth 20 mg orally. Venous blood was sampled from an indwelling cannula in the left foreleg at 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, 480, 600, 720, 1440, 2160 and 2880 minutes. The samples were transferred to Vacutainer^{R} tubes[10] containing heparin. In both studies, the animals were removed from their slings following the 240 minute sample and placed in metabolic cages until the completion of the study.

Plasma was separated from blood within one hour of collection. Plasma was then pipetted into one dram screw cap vials,[11] capped and stored at -20^{o}C. Additional blood specimens were collected to determine the plasma to whole blood concentration ratio. Usual diet was resumed 8 hours after dosing. Routine laboratory tests were within normal limits preceding and following the testing periods.

c. Assay procedure

Prazosin concentrations in plasma and whole blood were determined as described for Method I (Chapter III), with the inclusion of a Waters[12] Intelligent Sample Processor (WISP^{R}) for sample injection to the instrumentation.

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[11] Kimble Products Division, Owens-Illinois, Toledo, OH
[12] Waters Associates, Milford, MA

d. Calculations

The area under the plasma concentration-time curve (AUC_p) from time zero to the last data point was calculated by the linear-trapezoidal method to the first sample after the peak plasma concentration (C_{peak}), and thereafter by the log-trapezoidal method (146). The rate constant of elimination (λ_z) was calculated from the slope of the log-linear portion of the plasma concentration-time curve using the method of least squares. This value was then used to calculate the AUC from the last sample point to time infinity (147).

The absolute bioavailability (F), expressed as a percentage, was determined in each animal by comparing the dose-corrected areas under the plasma concentration-time curve following iv and po administration:

$$F = 100 \times (AUC_{po} \times Dose_{iv}) / (AUC_{iv} \times Dose_{po}) \quad \text{(IV-1)}$$

The relationship

$$CL_p = Dose / AUC_p \quad \text{(IV-2)}$$

was used to determine plasma clearance (CL_p) after intravenous dosing. The blood clearance (CL_B) was calculated by multipling the CL_p by the plasma:blood concentra-

tion ratio. Because sampling was frequent at the time of the peak concentration, the highest concentration measured is reported as C_{peak}, and the time of this sample is reported as the peak time (T_{peak}). The terminal half-life of prazosin was calculated by dividing ln 2 by the terminal rate constant of elimination. Lag-time (t_{lag}) was estimated by extrapolating the terminal log-linear phase and the feathered log-linear absorption phase, from the method of residuals, to time zero, and where the lines intersected at some point greater than t=0 was reported as the t_{lag}.

Assuming an hepatic blood flow (Q_H) of 31 ml/min per kg (148) and that the clearance does not change between doses, F, expressed as a percentage, was predicted by the equation

$$F/100 = 1 - E = 1 - CL_H/Q_H \qquad \text{(IV-3)}$$

where E represents the extraction ratio and CL_H represents the hepatic clearance (in this case clearance blood). The steady-state volume of distribution (V_{ss}) was calculated from the intravenous blood data by the non-compartmental method of Benet and Galeazzi (149) and corrected for infusion time (150).

Data are expressed as the mean $\pm$ standard deviation (SD) unless otherwise indicated. Tests for statistical differences were performed with the t-test for paired data.

2. Results

Plasma concentration-time curves from each of the four dogs following intravenous and oral dosing are found in Figures IV-1 and IV-2. Dosing and weight data for the dogs used in the study and the pharmacokinetic parameters determined from the iv data are found in Table IV-1, and from the po data in Table IV-2.

A comparison of the half-lives following oral and iv dosing (158 $\pm$ 32 and 192 $\pm$ 31 minutes) revealed a statistically significant difference ($p<0.05$). The steady-state volume of distribution and the whole blood clearance were 31.0 $\pm$ 2.6 liters and 6.95 $\pm$ 0.74 L/min, respectively. The mean predicted availability (Equation IV-3) in the four dogs was 73 $\pm$ 2% (range: 72-75%). The experimental bioavailability (Equation IV-1) was 77 $\pm$ 11%.

Following oral administration, a lag-time was apparent in three of the animals, as no detectable prazosin was found in the plasma prior to the 30 minute sample. Upon extrapolation of the terminal exponential phase and the feathered absorption curve to time zero, the intersection of these two lines yielded an estimate of t_{lag} which averaged 42 $\pm$ 34 minutes in the four animals. The plasma:whole blood concentration ratio was 0.57 $\pm$ 0.03.

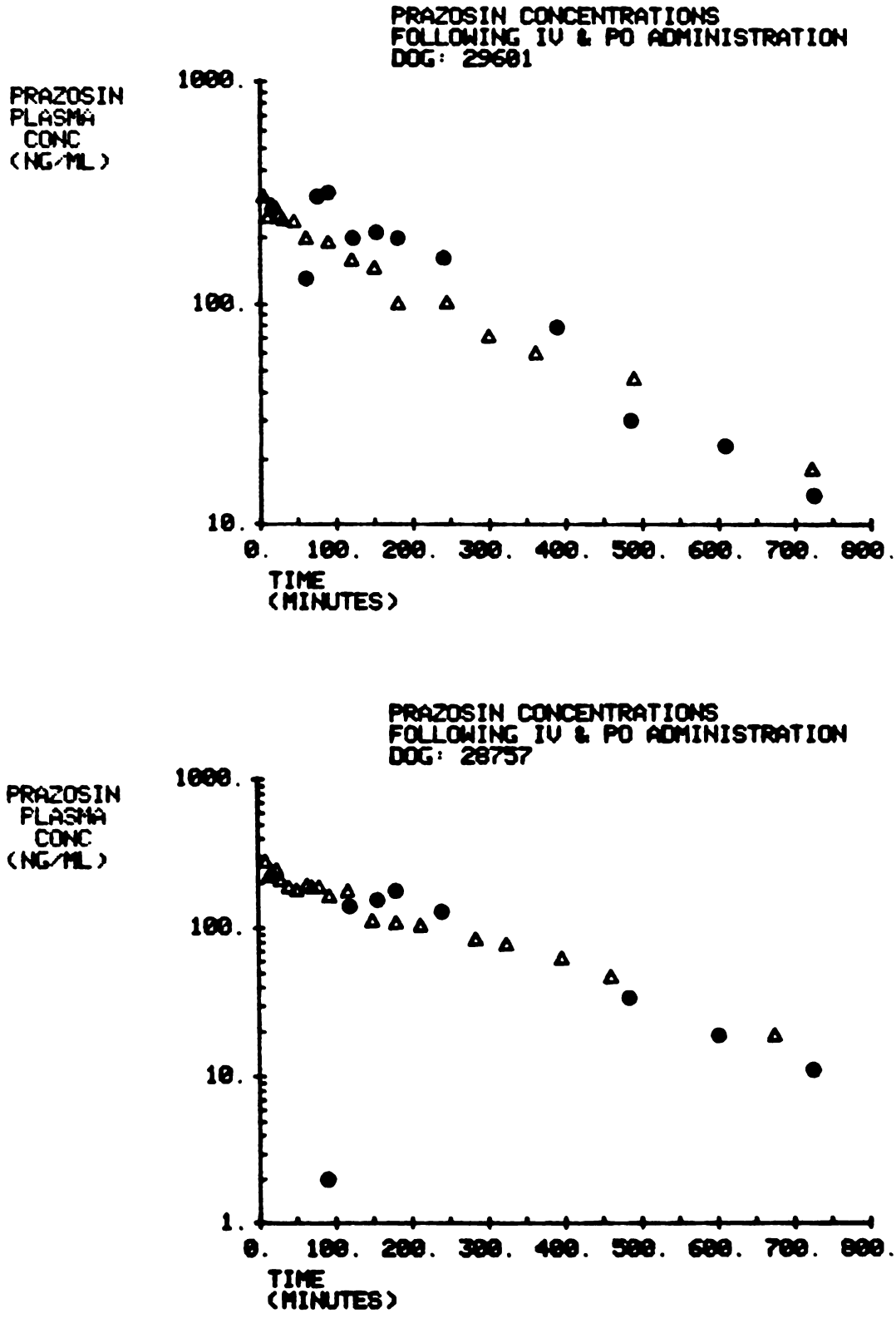

Figure IV-1. Plasma concentration time profiles for dogs 29601 and 28757 following intravenous (△) and oral (●) prazosin administration of approximately 1 mg/kg.

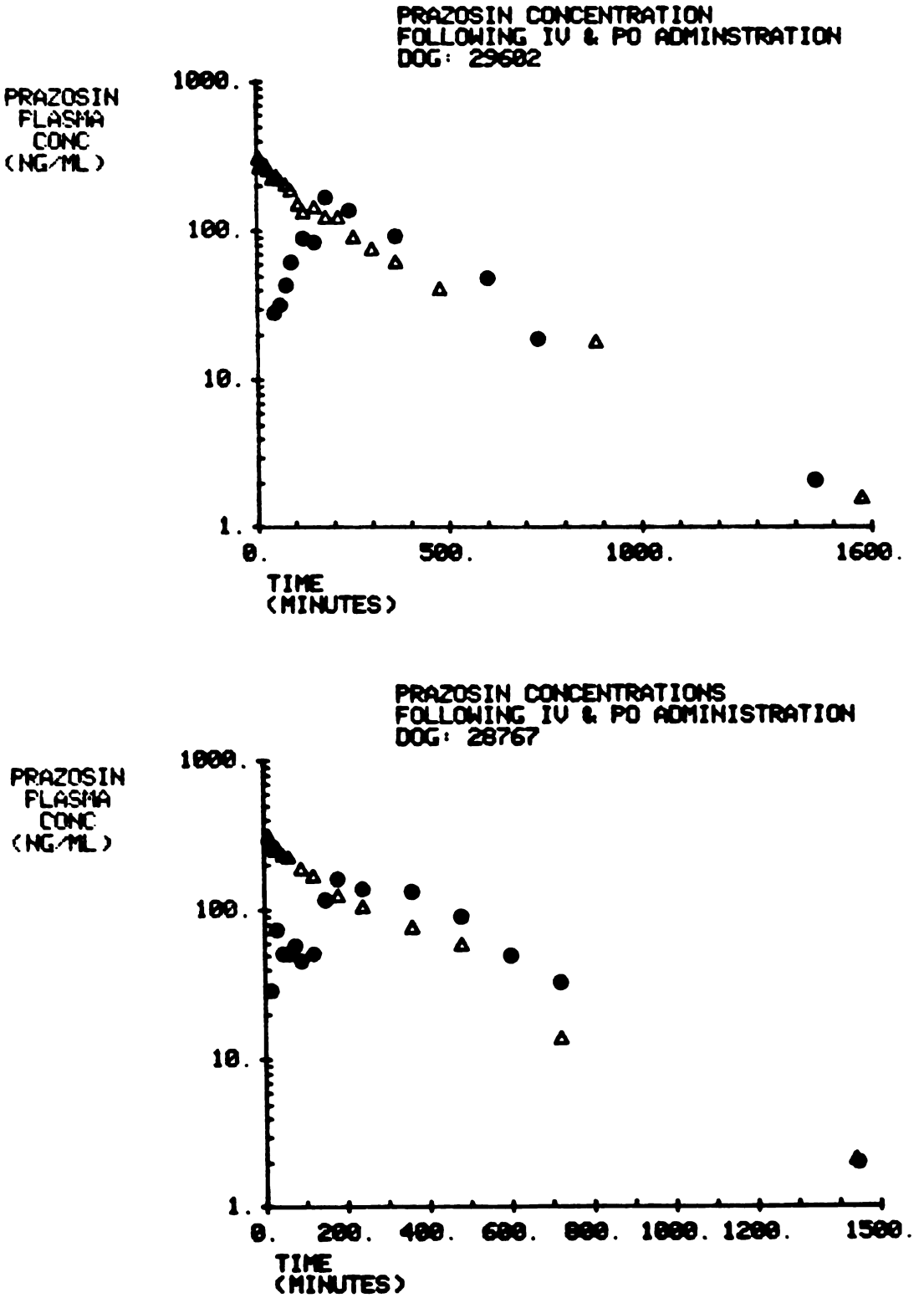

Figure IV-2. Plasma concentration-time profiles for dogs 29602 and 28767 following intravenous (△) and oral (●) administration of approximately 1 mg/kg.

Table IV-1. Animal data and pharmacokinetic parameters obtained following iv prazosin administration.

DOG #	WEIGHT (kg)	$Dose_{iv}$ (mg)	λ_z (min^{-1})	$t_{½z}$ (min)	V_{ss} (L)	$AUC_{0_P}^{\infty}$ (ng·hr/ml)	CL_B (L/hr)
28757	13.5	13.5	0.00433	160	30.4	1090	7.05
29601	12.5	12.5	0.00375	185	27.7	1140	6.23
29602	14.0	14.0	0.00295	235	32.3	1210	6.57
28767	16.5	17.2	0.00369	188	33.7	1240	7.93
MEAN	14.1	14.3	0.00368	192	31.0	1170	6.95
± SD	2.0	1.7	0.00057	31	2.6	70	0.74

Table IV-2. Animal data and pharmacokinetic parameters obtained following po prazosin administration.

DOG #	WEIGHT (kg)	$Dose_{po}$ (mg)	λ_z (min^{-1})	$t_{½z}$ (min)	t_{lag} (min)	$AUC_{0}^{\infty}{}_{P}$ (ng·hr/ml)	F_{pred}[a] (%)	F_{exptl}[b] (%)
28757	13.5	15[c]	0.00478	145	78	750	72	62
29601	12.5	15[c]	0.00554	125	54	1160	72	84
29602	14.0	15[c]	0.00345	201	36	990	75	76
28767	16.5	20[d]	0.00433	160	0	1230	74	85
MEAN	14.1		0.00453	158	42[e]	1030	73	77
± SD	2.0		0.00087	32	33	210	2	11

[a] Equation IV-3
[b] Equation IV-1
[c] three 5 mg capsules
[d] four 5 mg capsules
[e] n=4

3. Discussion

Prazosin, as commerically available 5 mg capsules, was administered orally and intravenously to four male beagle dogs at an approximate dose of 1 mg/kg. Previous studies on the cardiovascular effects of prazosin in dogs had indicated that this dose was handled well by the animals, and would generate plasma (and whole blood) concentrations that would be readily quantitated with the assay (Method I) described in Chapter III.

The half-lives after oral dosing (158 $\pm$ 32 minutes) were found to be significantly different statistically from the half-lives determined following intravenous administration (192 $\pm$ 31 minutes) in this study. This difference is not thought to be indicative of dissimilarities in the elimination of the compound due to the different routes of administration, but due to the small sample size and the greater number of measurable samples at later times following the intravenous dose. The volume of distribution steady-state was 31.0 $\pm$ 2.6 liters, which is representative of basic compounds and appears to be indicative of significant tissue distribution.

Following intravenous administration the whole blood clearance was 6.95 $\pm$ 0.74 L/min. This clearance value was used in predicting the bioavailability of an oral dose using Equation IV-3. Certain specific assumptions are required

for this prediction: 1) linear kinetics; 2) all of the compound (>90%) is cleared by the liver; and, 3) complete absorption with no gut wall metabolism. Deviations from linear kinetics have not been reported for prazosin, nor have there been reports of poor absorption or gut wall metabolism. Studies on the metabolic fate of prazosin in dogs (5) indicated that less than 6% of an oral dose is recovered unchanged in the urine. Thus, the assumptions necessary for predicting the extraction ratio appear valid.

The estimate for hepatic blood flow in dogs was obtained from experiments where flow was measured by electromagnetic flowmeters or venous long circuits without cannulation of the hepatic artery. These methods are thought to give the most accurate measurements of hepatic flows. The mean reported value for the total hepatic blood flow in conscious dogs is 31 ml/min per kilogram (148).

Using this estimate for hepatic flow in the dogs, a mean predicted bioavailability of 73 $\pm$ 2% was obtained. The experimental bioavailability (calculated by dose-corrected areas) following oral administration was 77 $\pm$ 11% (range: 62-85%). Thus, the predicted availability corresponds quite well with the calculated experimental value. This would appear to indicate that the assumptions used in predicting F are valid, especially the supposition of complete absorption with no gut wall metabolism as no data were available in this area.

Interestingly, the predicted bioavailability and experimental absolute bioavailability are greater than that reported by Rubin et al. (111). These investigators determined a mean F of 38 ± 11% following the oral administration of 5 mg prazosin in a gelatin capsule and 1 mg iv to three conscious mongrel dogs (mean weight: 22.6 kg). These doses are much smaller than the dosages used in this study, which were standardized to body weight. The half-lives obtained in both studies compare favorably, as do the blood clearances and volumes of distribution steady-state when normalized to weight (see Table IV-3). The most obvious difference in the data is in the comparison of the experimental availabilities (38 to 77%).

The difference in bioavailability between the two studies may be explained by a number of factors. First, random source mongrel dogs may handle drugs differently than laboratory bred beagles. The prediction of F was based on an estimate of liver blood flow of 31 ml/min per kg, which is greater than that which would be back calculated in Equation IV-3 from the data of Rubin and coworkers (111), approximately 17 ml/min per kg, which is below any estimate for canine hepatic flow (148). Unpublished preliminary data[13] indicated that the clearance of quinidine in mongrel dogs was greater than in beagles, but this observation has not been substantiated.

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[13]T. Guentert, personal communication, 1980

Table IV-3. Comparison of pharmacokinetic parameters in the dog.

	Rubin et al. (n=3)	This study (n=4)
WEIGHT (kg)	22.6	14.1 ± 2.0
ORAL DOSE (mg/kg)	0.22	1.15
$t_{½_{po}}$ (min)	153 ± 24	158 ± 32
CL_B (ml/min/kg)	10.3 ± 2.5	8.23 ± 0.9
MEAN V_{ss} (L/kg)	2.15	2.24
$F_{predicted}$[a] (%)	67	73
$F_{experimental}$ (%)	38 ± 11	77 ± 11

Values reported as MEAN (± SD)

[a]Assuming an hepatic blood flow of 31 ml/min/kg (148)

Secondly, the difference in oral doses (0.22 vs 1.15 mg/kg) and the availability at these doses suggests a possible saturation of some presystemic metabolic mechanism. Thus, the necessary assumptions that accompany the treatment of first-pass metabolism may be incorrect, as it appears as though not all of the dose was absorbed and/or gut wall metabolism occurred. Small amounts of an oral dose may be metabolized in the gut wall and this mechanism may become saturated as the oral dose is increased. The assumptions of first-pass metabolism occurring exclusively in the liver would then be functionally incorrect, and as the oral doses vary, differences in the predicted and experimental availabilities would be evident. Thus, it would appear necessary to determine the bioavailability of low, medium and high oral doses of prazosin in beagle dogs to more closely examine the discrepancy cited above.

4. Summary

Experimental evidence in beagle dogs indicates that based on the assumptions for predicting availability, the dose administered and the estimate of Q_H, the experimental absolute bioavailability of oral prazosin is in good agreement with the predicted F. Thus, prazosin appears to be relatively well absorbed at this dosage, but the difference evident at lower oral doses reported by others (111) may

indicate the existence of saturable pre-hepatic metabolic processes.

C. Dose Dependent Prazosin Bioavailability in Beagle Dogs

1. Experimental

a. Specific objective

To determine if the difference in the bioavailabilities between the results presented in Section B and those of Rubin et al. (111) was due to the large difference in the oral doses administered (1.15 vs 0.22 mg/kg), the bioavailability of prazosin hydrochloride was determined following three oral doses (1, 5 and 15 or 20 mg).

b. Study design

Three male beagle dogs (#: 29601, 29602 and 28767) from the previously described prazosin bioavailability and disposition study (Section B), as well as data obtained in that study, were used. Following an overnight fast, animals were administered a single 5 mg oral prazosin capsule (lot #: 85110). After a 7-day washout interval, prazosin was dosed as a single 1 mg oral capsule (lot #: 98122). Immediately

following each oral dose the animals received 30 ml of distilled water, and were offered water ad libitum throughout the study period. All other experimental conditions were identical to those described in Section B.1.b.

c. Assay procedure

Prazosin concentrations in plasma and whole blood were determined using Method II (Chap.III), with tiodazosin as the fluorescent internal standard. Different standard curves were constructed for the two dosages. The calibration curves for samples taken following the 5 mg oral dose were constructed over the concentration range of 0.9 to 46 ng/ml (see Figure III-6), and low dose (1 mg) calibration curves were prepared from 0.2 to 11.5 ng/ml (see Figure III-7).

d. Calculations

All pharmacokinetic parameters were calculated as discussed in Section B. Statistical analyses of the dose-normalized areas under the plasma concentration-time curves and the bioavailabilities determined following the three oral doses were performed using the one-way analysis of variance (ANOVA).

2. Results

The data from the 1 and 5 mg oral dose studies were compared to the intravenous and 15 or 20 mg oral dose data described in Section B. Figure IV-3A is a representative plasma concentration-time curve (dog #: 29602) following the iv administration of 1 mg/kg. Plasma concentration-time curves obtained after the iv dose showed 2-compartment body model characteristics. The plasma profiles obtained in the same animal following the three different oral dosages are presented in Figure IV-3B.

Information on the size of the animals, the dose administered and the pharmacokinetic parameters determined from the iv dosing are given in Table III-3. The mean values for the clearance and steady-state volume of distribution were 6.95 $\pm$ 0.74 L/min and 31.0 $\pm$ 2.6 L/min, respectively. The mean predicted availability based on the 1 mg/kg iv data was 74 $\pm$ 2% (range: 72-75%). Prazosin area measurements and the experimental availability following the three different oral doses are presented in Table IV-4. The mean bioavailabilities for the 15, 5 and 1 mg doses were 82, 27 and 23%, respectively. The plasma:whole blood concentration ratio (0.55 $\pm$ 0.09) was not different from the value determined at the higher dose (0.57 $\pm$ 0.03).

Although there was good agreement in bioavailability following the 15 mg dose with what was predicted, the

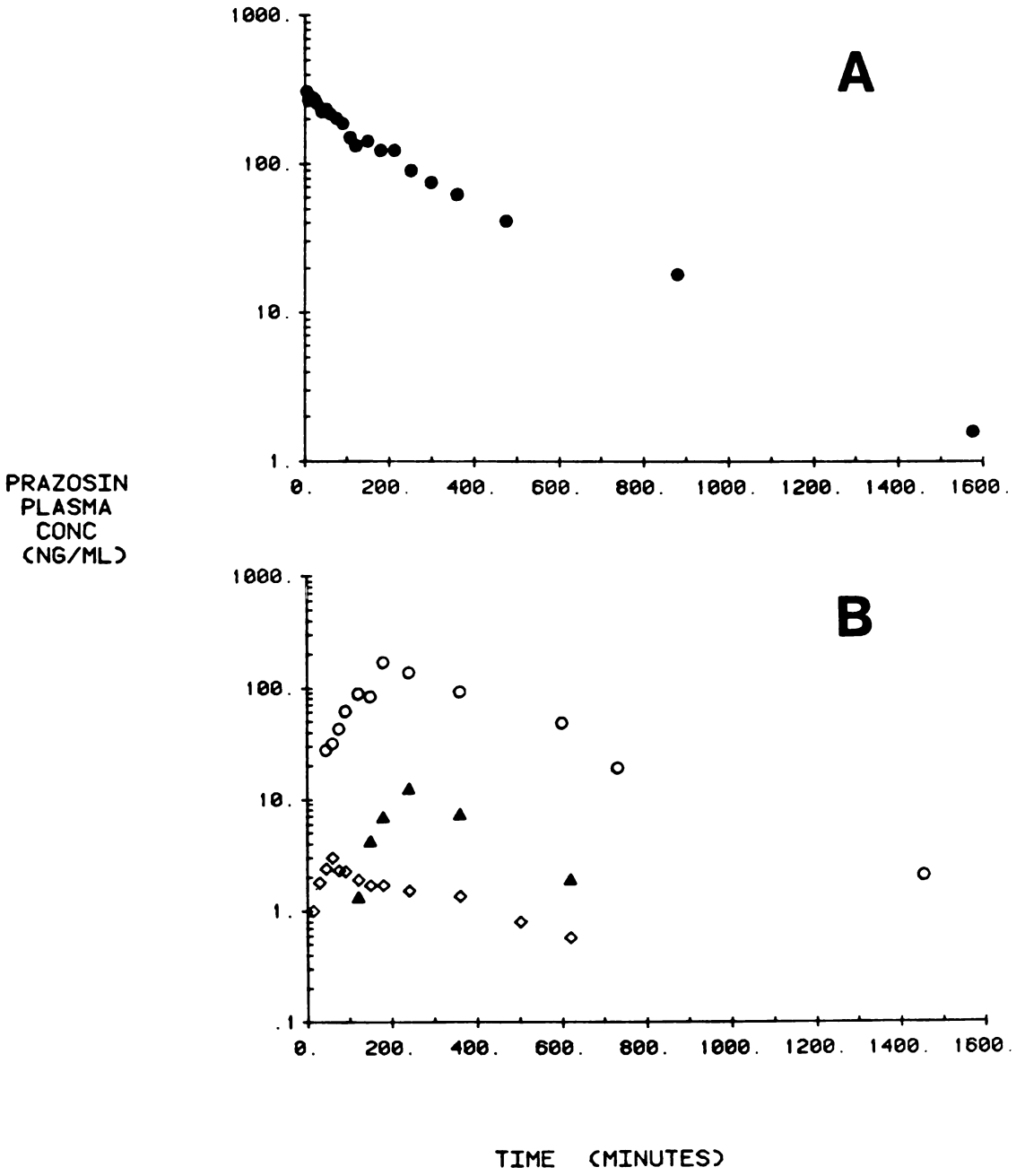

Figure IV-3. Prazosin plasma concentration-time profiles in a beagle dog (29602) following: A) intravenous dose (●) 1 mg/kg; B) oral doses - (○) three 5 mg capsules, (▲) a single 5 mg capsule, and (◇) a single 1 mg capsule.

Table IV-4. Prazosin area measurements and bioavailability calculations for 3 different oral doses.

DOG #	AUC_{iv} [a]	$\frac{AUC_{iv}}{Dose}$	AUC_A	$\frac{AUC_A}{Dose}$	F (%)	AUC_B	$\frac{AUC_B}{Dose}$	F (%)	AUC_C	F (%)
29601	1144	91.5	1151	77.0	84	98.6	19.7	22	19.2	21
29602	1215	86.8	992	66.1	76	59.9	12.0	14	15.9	18
28767	1237	71.9	1230[b]	61.5	85	160.	32.0	45	22.7	31
MEAN	1199	83.4		68.2	82	106.	21.2	27	19.3	23
± SD	49	10.2		8.0	5	50.	10.1	16	3.4	7

A = 15 mg; B = 5 mg; C = 1 mg Area comparison (ANOVA) yielded a $p<0.002$

[a] AUC units are μg·hr/ml; since the oral dose C is 1 mg, a dose-corrected area is not presented as a separate column in the Table.

[b] This animal received an oral dose of 20 mg (4 capsules).

experimental availabilities from the 5 mg and 1 mg capsules were approximately one-third the predicted value. There is a significant difference ($p<0.002$, ANOVA) when the bioavailabilities are statistically compared (high vs med and high vs low doses).

The terminal half-lives determined for each treatment in each animal are found in Table IV-5. There is no statistically significant difference between the four groups.

3. Discussion

The results presented here indicate that the bioavailability of prazosin hydrochloride in beagle dogs is dose-dependent. The mechanism(s) for this observation remain(s) speculative. In man the bioavailability of prazosin from 1 mg tablets (free base equivalent) averaged 57% (117). Under the assumption that prazosin metabolism occurs exclusively in the liver, the hepatic extraction ratio was calculated to be 27% (Equation IV-3). The authors (117) concluded that although much of the reduction in F following oral administration may be attributable to "first-pass" elimination, it would appear that other factors may also contribute. These other factors would include extrahepatic metabolism and incomplete gastrointestinal (gi) absorption. Rubin *et al.* (111) studying mongrel dogs found a mean hepatic extration ratio of 47% which would predict an oral availability of

Table IV-5. Half-lives[a] in all animals from the four different prazosin dosings (iv and po).

DOG #	$t_{\frac{1}{2}z}^{iv}$ (min)	$t_{\frac{1}{2}z}^{A}$ (min)	$t_{\frac{1}{2}z}^{B}$ (min)	$t_{\frac{1}{2}z}^{C}$ (min)
29601	185	125	149	207
29602	235	201	143	182
28767	188	160	166	202
MEAN	203	162	153	197
± SD	28	38	12	13

A = 15 mg; B = 5 mg; C = 1 mg

[a]no statistical difference (ANOVA)

53%, assuming that presystemic removal of the drug occurred exclusively in the liver. The fact that their experimental F was considerably lower (38%) also suggested that some other factors, such as gut wall metabolism or incomplete gi absorption, were contributing to the low availability in dogs.

In the experiment discussed in Section B, an oral dose of approximately 1 mg/kg was used, which is higher than the dose used by Rubin and coworkers (111). Using Equation IV-3 to predict oral availability, good agreement was found with what was experimentally determined in four beagle dogs. In the experiment presented here, smaller doses of prazosin did not give the same F as predicted, but availabilities that were 2 to 3 times lower. These values are in relatively good agreement with the dog study using a smaller dose (111).

Differences in the gastrointestinal tract between man and dogs precludes any firm extrapolation of the data at this time. But, the data presented here would appear to indicate that the dose-dependent oral availability in dogs is the result of alterations in some saturable metabolic process(es). A possible explanation would be some form of gastrointestinal metabolism, such that at high doses this mechanism is saturated and more drug is absorbed from the gi tract and presented to the liver. At this high dose, the availability would tend to agree with what is predicted

based on exclusive "first-pass" metabolism. At lower doses however, saturation of the presystemic mechanism does not take place, a greater percentage of the compound is acted upon before reaching the liver and a lower than predicted bioavailability is the result. A dose-dependent prazosin clearance is not thought to be the case, for two reasons: 1) the CL values determined by Rubin et al. (111) following a 1 mg iv dose are comparable to those values determined in Section B, where a 15-fold greater intravenous dose was administered, and 2) the half-lives at all doses, whether iv or oral, did not show a statistically significant difference. It is also possible that some incomplete dissolution of the dosage form may also contribute to the variable bioavailability of this agent. Excipients that promote dissolution may be more effective following multiple capsule dosing than following the oral administration of a single capsule.

4. Summary

From the results presented here, together with the data of Rubin and coworkers (111), it would appear that the bioavailability of prazosin hydrochloride in dogs is dose-dependent.

CHAPTER V

ALTERED PRAZOSIN PHARMACOKINETICS IN CONGESTIVE HEART FAILURE

A. Background

In the last several years, clinical trials have shown prazosin to be of value in the treatment of chronic congestive heart failure (CHF) (77-83). Data from these studies indicate that prazosin may reduce cardiac pre-load and after-load through dilation of both capacitance and resistance vascular beds.

Pharmacokinetic investigations in healthy individuals have indicated that the systemic bioavailability of prazosin is approximately 60% and that the drug is eliminated almost exclusively by the liver.

Congestive heart failure may be associated with a reduction in hepatic clearance for drugs that are eliminated primarily via hepatic metabolism (129,151,152). The reduction in drug elimination and the subsequent increase in plasma or blood drug concentrations at a given dose has been attributed to a reduction in hepatic blood flow for drugs with a high hepatic extraction ratio, or to a reduction in intrinsic hepatic clearance for drugs with a low extraction ratio. Assuming that the pharmacologic effects of prazosin in the therapy of hypertension and CHF are similar, and assuming that there is a relationship between plasma (or

blood) drug concentrations and effect, these kinetic observations would suggest that the dose needed to treat individuals with CHF should be lower than the dose used to treat individuals with hypertension. Clinical trials of prazosin in congestive heart failure patients have shown, however, that the amount of drug that is initially required to achieve hemodynamic and clinical response is higher than the dose used initially in the treatment of hypertension.

B. Specific Objective

To determine if plasma prazosin concentrations are higher in CHF patients than in normals, the disposition of prazosin following the oral administration of a 5 mg capsule was studied in individuals with chronic congestive heart failure and in healthy controls.

C. Experimental

1. Patient selection

Nine patients (three female and six male) with New York Heart Association Class 3 or 4 congestive heart failure participated in the pharmacokinetic study. Five healthy individuals (three female and two male) participated in the study as controls. Mean age for the patients in the study

was 62 years (range: 49-77) and was 39 years (range: 32-50) for the healthy controls. Although the study protocol was designed to include an equal number of patients and controls (age- and sex-matched) the study was discontinued in the control group after entry of the fifth subject because of prominent orthostatic hypotension appearing in three of the five healthy individuals. Mean ($\pm$ SD) weight in the patient group was 69.0 $\pm$ 15.2 kg and in the healthy subjects was 75.0 $\pm$ 12.7 kg. All participants were admitted to the hospital for the duration of the study after they were fully informed of the nature and risks of this investigation, and their informed consent was obtained. Patients were hospitalized in a coronary care unit.[1] Each of the patients in the study received their standard maintenance dosages of digitalis and diuretic preparations throughout the study period of prazosin dosing and sample collection. The etiology of congestive heart failure in the nine patients was as follows: coronary artery disease - 7 patients; hypertensive cardiomyopathy with coronary artery disease - 1 patient; and, cardiomyopathy and diabetes mellitus - 1 patient. Healthy subjects took no medication for at least ten days prior to the onset of the study. Smoking was not permitted. Liver function tests, blood urea nitrogen (BUN), serum creatinine and urinalysis in both healthy and patient groups were within normal limits.

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[1]Moffitt Hospital, University of California, San Francisco

2. Study design

Following an eight hour fast, each of the 14 subjects in the study received 5 mg of prazosin[2] (lot #: 60232) orally with 250 ml water. Relative to the time of this dose, blood samples were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours. Blood samples from patients were drawn through a pulmonary artery catheter. Blood samples in healthy control individuals were drawn through an indwelling venous line[3] placed in a forearm vein. Patency of this line was maintained by periodic injections of small amounts of heparin in saline. Plasma was separated from blood within one hour of collection and stored at -20°C. Additional blood specimens were collected at 2 and 8 hours to determine the plasma to whole blood concentration ratio. Urine was collected for drug assay over the 24 hours following dosing. Participants in the study remained fasting until four hours after dosing, at which time they were given a liquid lunch. Usual diet was resumed 8 hours after drug administration. Healthy subjects in the study remained recumbent except for periodic determination of standing blood pressure.

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[2]Minipress[R] - Pfizer Inc.

[3]Butterfly[R] INT 21G X 3/4" Intermittent Infusion Set, Abbott Hospitals, North Chicago, IL

3. Assay procedure

Prazosin concentrations in plasma, whole blood and urine were determined by Method I as described in Chapter III. Representative chromatograms for plasma (Figure III-4) and urine (Figure III-5), obtained from patients in this study, are presented in Chapter III. Calibration graphs were constructed from spiked plasma over the concentration range of 2 - 76 ng/ml (see Figure III-3).

4. Calculations

The following pharmacokinetic parameters were calculated from the plasma concentration-time curve obtained in each individual: area under the plasma concentration-time curve (AUC), peak plasma concentration (C_{peak}), time of peak plasma concentration (T_{peak}), rate constant of elimination (λ_z) and half-life. AUC was calculated by the linear trapezoidal method to the C_{peak} and thereafter by the log-trapezoidal method (146). C_{peak} and T_{peak} were determined by visual inspection. The rate constant of elimination was calculated from the slope of the log-linear portion of the plasma concentration-time curve using the method of least squares. In the event this portion of the curve was not log-linear at the time sampling was stopped, the final two points of the concentration-time curve were used to estimate

the elimination rate constant. This value was then used to calculate the AUC from the final sampling point to time infinity (147). The half-life of prazosin was calculated as the ln 2 divided by the rate constant of elimination. The plasma clearance divided by the availability of the oral dose (CL_P/F) was calculated by dividing the dose by the AUC. Multiplying this value by the plasma:blood concentration ratio of the drug yielded blood clearance divided by oral availability (CL_B/F). Absolute bioavailability of prazosin in this experiment could not be determined since an intravenous preparation of the drug for use in humans is lacking.

Comparisons between pharmacokinetic parameters for the patient and control groups in the study were made using the t-test for unpaired data.

D. Results

Plasma concentration-time curves for the patients and controls in the study, averaged at each sampling time, are shown in Figure V-1. It is apparent from these average values that the peak plasma prazosin concentration (C_{peak}), area under the plasma concentration-time curve (AUC) and the terminal half-life were increased in individuals with chronic congestive heart failure. Concurrent medications in the patient group were shown not to interfere with the pra-

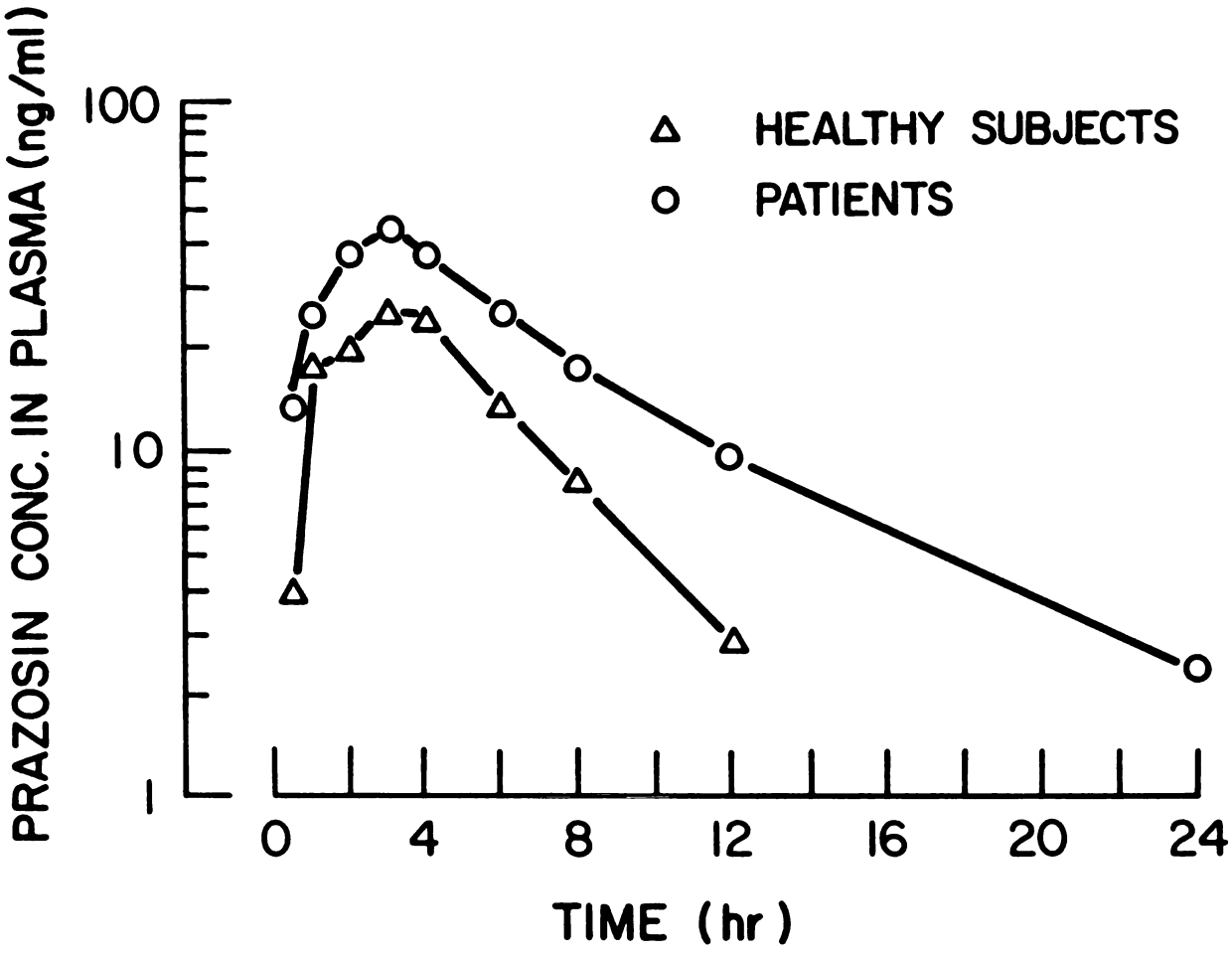

Figure V-1. Prazosin concentrations in plasma following oral administration of 5 mg to 9 individuals in congestive heart failure and to 5 healthy control subjects. Average values at each sampling time are shown.

zosin assay. Pharmacokinetic parameters calculated from individual plasma concentration-time curves are shown in Table V-1. Statistical analysis of these data confirm that prazosin AUC increased significantly ($p<0.02$) and CL_B/F and the rate constant of elimination decreased significantly ($p<0.05$) in the individuals with congestive heart failure in comparison to the healthy controls. Average half-life in the patient group was more than twice that observed in the healthy individuals (6.47 ± 4.55 vs 2.55 ± 0.43 hours). T_{peak} and the plasma:blood concentration ratio did not differ significantly between the two groups. Although not significantly different, the peak plasma prazosin concentration in the patient group was substantially higher than in the healthy controls. The fraction of the dose excreted unchanged in the urine was low in both the patient and control groups (<2%) and did not differ significantly between them (see Table V-2). The presence of unchanged drug in the 24-48 hour urine collection in some of the normal subjects (Table V-2) suggests, although not strongly, the presence of a deep tissue compartment.

E. Discussion

This study demonstrates that plasma (and blood) concentrations of prazosin, as assessed by area under the plasma concentration-time curve, may be significantly higher in

Table V-1. Pharmacokinetic parameters determined in 5 healthy and 9 patient volunteers receiving a single 5 mg oral dose of prazosin.

SUBJECT	C_{peak} (ng/ml)	T_{peak} (hr)	λ_{z_1} (hr^{-1})	$t_{½z}$ (hr)	$AUC_{0_P}^{\infty}$ (ng·hr/ml)	P:WB[a] Ratio	CL_B[b] (ml/min)
HEALTHY VOLUNTEERS							
1A	22.1	1	0.217	3.19	122	1.36	932
2A	14.6	3	0.283	2.45	77	1.70	1850
3A	35.0	1	0.347	2.00	120	1.28	887
6A	49.1	3	0.267	2.60	233	1.31	469
7A	42.2	4	0.277	2.50	222	NA[c]	529[d]
MEAN	32.6	2.4	0.278	2.55	155	1.41	933
± SD	14.2	1.3	0.046		69	0.19	551
PATIENT VOLUNTEERS							
1	87.0	4	0.086	8.10	518	1.30	209
2	77.1	3	0.108	6.42	537	1.45	225
3	45.7	2	0.100	6.93	323	1.52	393
4	42.5	3	0.231	3.00	274	1.28	390
5	52.3	1	0.161	4.30	643	1.58	205
7	33.0	3	0.087	7.97	253	1.35	446
8	60.3	2	0.385	1.80	248	1.47	495
9	46.4	2	0.245	2.83	220	1.79	679
10	8.9	3	0.041	16.9	158	1.58	832
MEAN	50.4	2.6	0.161[e]	6.47	353[δ]	1.49	430[e]
± SD	23.1	0.9	0.109		169	0.16	216

[a] Plasma:Whole blood concentration ratio; mean of two determinations, at 2 and 8 hours post-dosing.

[b] Assuming complete absorption without transformation into the portal vein

[c] Not available

[d] Intrinsic clearance calculated using mean P:WB ratio for volunteers 1A, 2A, 3A and 6A.

[e] $p<0.05$ [δ] $p<0.02$

Table V-2. Renal elimination of prazosin.

SUBJECT	COLLECTION TIME (hr)	CONCENTRATION (ng/ml)	URINE VOLUME (ml)	AMOUNT (μg)	PERCENT OF DOSE EXCRETED UNCHANGED
HEALTHY VOLUNTEERS					
1A	0 - 24	9.1	955	8.7	0.2
	24 - 48	0	840	0	0
2A	0 - 24	3.0	2970	8.8	0.2
	24 - 39	2.6	410	1.1	>0.1
3A	0 - 24	17.1	1415	24.2	0.5
	24 - 48	0	2040	0	0
6A	0 - 24	17.6	2500	44.0	0.9
	24 - 48	0	NA[a]	0	0
7A	0 - 24	18.9	2910	55.0	1.1
	24 - 48	2.5	2898	7.2	0.1
PATIENT VOLUNTEERS					
1	0 - 24	6.6	1660	11.0	0.2
2	0 - 24	30.3	445	13.5	0.3
3	0 - 24	25.3	1060	26.7	0.5
4	0 - 24	2.4	1625	3.8	0.1
5	0 - 24	32.9	1050	34.5	0.7
7	0 - 24	8.9	1630	14.5	0.3
8	0 - 24	7.2	1695	12.2	0.2
9	0 - 24	7.7	3472	26.8	0.5
10	0 - 24	4.5	1543	6.9	0.1

[a]Not available

patients with chronic congestive heart failure than in healthy individuals following the administration of an identical oral dose of the drug. Although some overlap in the pharmacokinetic parameters between the two groups was apparent, there was a statistically significant difference between patient and control populations for both AUC and λ_z. No difference in the pharmacokinetic parameters between males and females was noted in the CHF group. The average half-life in the patient group was more than twice that observed in the control population. These observations suggest that steady-state plasma prazosin concentrations may be higher in patients with chronic congestive heart failure and that the time required to reach steady state may also be increased. Assuming that steady-state plasma concentrations are attained in approximately three to four drug half-lives, the time required to reach steady state may increase to 24 hours or more following initiation of a stable dosing regimen in patients with CHF, as compared to the approximate 10 hours required in healthy controls.

Due to the occurrence of prominent orthostatic hypotension in the healthy individuals, the experiment was terminated in the control group after studying the fifth subject. Comparison with other studies in normal individuals receiving the same dose in the same dosage form (see Table V-3), indicated that the pharmacokinetic data from this study was representative of the normal population.

Table V-3. Comparison of pharmacokinetic parameters from four separate investigations where normal subjects received a single 5 mg oral prazosin capsule.

Author/ Ref	# of Subjects	C_{peak} (ng/ml)	T_{peak} (hr)	AUC_P (ng·hr/ml)	$t_{½}$ (hr)
This work	5	32.6	2.4	155	2.55
Hobbs et al. (113)	24	35.9	2.2	174	2.66
Jaillon et al. (118)	10	NR[a]	2.1	188[b]	2.42
Pitterman et al. (121)	5	29.3	1.0[c]	174	2.9

[a]Not reported

[b]AUC values were reported for blood data (AUC_B=134 ng·hr/ml); the number presented here represents that value multiplied by the plasma:blood ratio (1.41) determined in this study.

[c]Sampling times were at 0, 0.5, 1, 3, 5 and 7 hours only.

Two studies with similar objectives have been reported. Jaillon et al. (118) determined the pharmacokinetics of prazosin in nine CHF patients and in 10 healthy controls. The controls (mean age: 27 years) received a 5 mg oral prazosin capsule, whereas the patient group (mean age: 57 years) received a 2 mg oral capsule. Following the single oral dose, whole blood samples were taken serially over 8 hours in the controls and over 10 hours in the patients. The t_{peak} and dose-normalized AUC were determined in each group and the half-life was calculated for the controls. The CHF group then received multiple oral doses of 2 to 5 mg (dose determined by blood pressure and heart rate) every 8 hours for two days. Whole blood was sampled serially over 24 hours following the last oral dose and a weighted fit of the log-linear terminal phase was used to calculate the half-life in this group. The pharmacokinetic parameters reported from this study, found in Table V-4, are in good agreement with the results presented here.

Silke et al. (127) measured the plasma concentrations in samples taken at 0, 0.5, 1, 2, 4 and 8 hours following four different oral prazosin doses (0.5, 1, 2 and 4 mg) to CHF patients. Within patient variability was also determined in this study by measuring plasma concentrations in samples taken from four patients who received the same oral prazosin dose on three successive days. Only 5 of the 8 patients were able to tolerate doses greater than 1 mg due

Table V-4. Comparison of mean pharmacokinetic parameters from three separate investigations of prazosin disposition in individuals with chronic congestive heart failure.

Author/ Ref	# of Subjects	Dose (mg)	C_{peak} (ng/ml)	T_{peak} (hr)	AUC_P (ng·hr/ml)	$\frac{AUC_P}{Dose}$ [a]	$t_{1/2}$ (hr)
This work	9	5	50.5	2.6	353	70.6	6.5
Jaillon et al. (118)	9	2	NR[b]	2.2	166[c]	83	6.2
Silke et al. (127)	8	0.5	6.2	2[d]	38	76	5.5
	8	1	9.4	2[d]	64	64	5.3
	5	2	20.3	2[d]	169	84.5	5.6
	5	4	37.1	2[d]	254	64	5.9

[a] units are ng·hr/ml·mg

[b] Not reported

[c] AUC values were reported for blood data (AUC=111 ng·hr/ml); this number represents that value multiplied by the plasma:blood ratio (1.49) determined in this study.

[d] Samples taken at 0, 0.5, 1, 2, 4 and 8 hours only.

to severe postural hypotension. The grouped results at each of the four doses are presented in Table V-4. These investigators infrequently sampled the plasma for only 8 hours, which is approximately 1.4 half-lives. Considerable day-to-day variation was reported in the plasma concentration-time profile of each of the four patients who took the same dose on three successive days. The variability was greatest at the 0.5 hour sample ("coefficient of variance": 27 - 50 %) and least at the 8 hours sample [CV (%): 8 - 14] over the four doses. These results may be confounded by residual prazosin in the systemic circulation from each preceding dose. The results reported from this study (excluding the half-lives) are quite variable and appear to be the result of too few plasma samples and/or a variable absorption rate constant.

These authors report that the differences seen between patients and normal controls are most likely due to tardy intestinal absorption and reduced liver metabolism that frequently accompany severe heart failure. Further, they speculate that the pharmacokinetic profile for patients in heart failure may be distorted by the competing actions of $alpha_1$-adrenoceptor blockade on liver function, that is, blockade of vasoconstriction in splanchnic vessels may be offset by a reduction in hepatic arterial perfusion pressure. Unfortunately, neither of these mechanisms is readily assessed and the contribution of α-blockade to pharmacok-

inetic alterations in heart failure is unknown.

Because an intravenous preparation of the drug was not available for administration to individuals in this study, or in the studies of Jaillon et al. (118) and Silke et al. (127), the reason(s) for the observed increase in plasma prazosin concentrations in the patient groups was not defined. Determination of drug clearance requires parenteral administration of the drug unless the assumption is made that the drug is entirely available to the systemic circulation following the oral dose. Available data in normals (117,120) would indicate, however, that this is not a valid assumption.

If it is assumed that all of the drug is absorbed from the gastrointestinal tract with no gut wall metabolism, and that there is a negligible difference in bioavailability between CHF patients and normals, then it is apparent from Table V-1 that the blood clearance of prazosin in CHF patients was reduced an average of approximately one-half in comparison to the clearance observed in the control group. These assumption are supported by the relationship

$$CL_B/F = f_u \times CL_{int}/F \qquad (V\text{-}1)$$

where f_u is the percent of the drug unbound and the intrinsic hepatic clearance, CL_{int}, is the maximal ability of the liver to irreversibly remove drug by all pathways in the

absence of any flow limitations. Rubin and Blaschke (133) have shown that the free fraction of prazosin is not significantly different in CHF patients as compared to normals (see Table II-4). Thus, the two-fold reduction in the values of f_u X CL_{int}/F (reported as CL_B/F in Table V-1) would appear to be due to a decrease in the CL_{int} (a decreased liver function). A reduction in the volume of distribution and an increase in the oral availability would also appear to contribute to the pharmacokinetic alterations observed in the patient group. Only a reduced f_u X CL_{int} can account for all the parameter changes seen in Table V-1, but the contribution of a reduced volume of distribution and/or an increased F cannot be excluded. Thus, in the absence of direct measurements of prazosin clearance, hepatic extraction or hepatic blood flow in the individuals in this study, conclusions about the mechanisms for the observed change in plasma prazosin concentrations in patients with chronic congestive heart failure remain speculative.

The results would indicate that the requirement for higher doses of prazosin in patients with congestive heart failure in comparison to individuals with hypertension does not arise from impaired absorption of the drug. Although many individuals with congestive heart failure appear to tolerate well these higher doses of prazosin, the finding of higher drug concentrations in these individuals together

with reports of significant orthostatic hypotension during initial dosing of the drug (97), indicate that patients who are given prazosin for the treatment of CHF should be closely monitored during the initial period of dosing for evidence of orthostatic hypotension and other adverse reactions.

CHAPTER VI

ANALYTICAL METHODOLOGY FOR CHARACTERIZING PRAZOSIN METABOLITES

A. Background

As discussed in Chapter II Section D, information on the metabolism of prazosin, especially in humans, is incomplete. What is known is that dogs and rats metabolize >96% of the parent compound to six metabolites (5), and three of the metabolites formed in the dog are pharmacologically active (4). The activity of these metabolites are reported to range from 10 to 25% of the parent compound (4). In humans, prazosin metabolites are not thought to be present in the blood (or plasma) (113,136). This contention is based on very limited experimental evidence and a great deal of extrapolation from animal data.

In the course of quantitating prazosin in plasma and whole blood samples from human subjects (Chapter V), the presence of time-dependent peaks was observed in the chromatograms (Method I - isocratic assay, Chapter II Section B.3) obtained from the plasma and whole blood samples of normal and congestive heart failure volunteers. Figure VI-1 presents only the fluorescence chromatograms in the isocratic analyses (Method I) of plasma and whole blood samples from a CHF patient following prazosin dosing. The heights

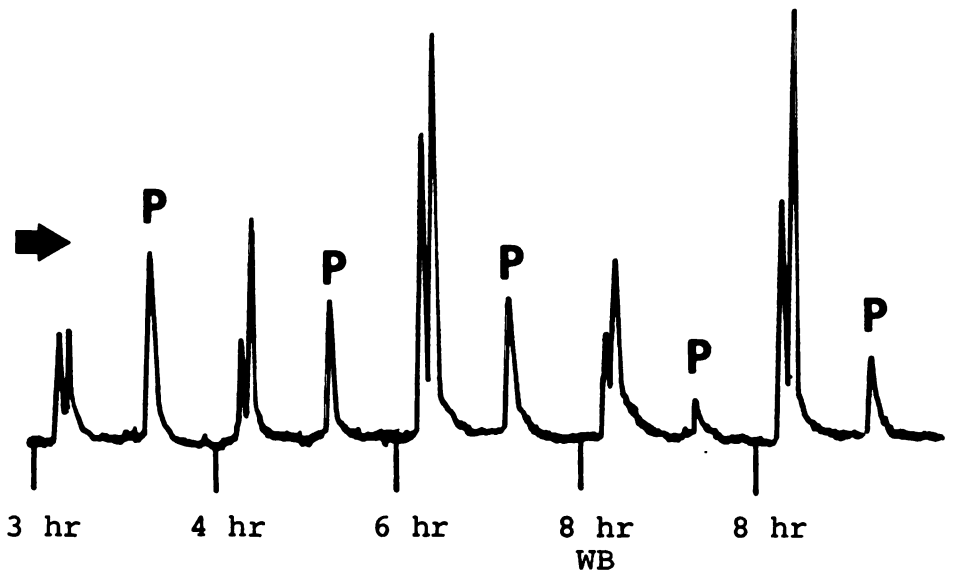

Figure VI-1. Time-dependent peaks in plasma and whole blood (WB) samples. Chromatograms are from the isocratic HPLC fluorescence prazosin assay of samples from a CHF patient. Only the fluorescence chromatogram from the assay is shown (Method I).

of the time-dependent (i.e., drug-related) peaks were measured, and the concentration-relative-to-prazosin was determined from the prazosin standard curve.[1] The "concentration"-time profiles generated for the time-dependent peaks, seen in Figure VI-2, have the appearance of the familiar concentration-time profile following drug administration or metabolite formation. The isocratic assay was performed with a reversed phase HPLC column,[2] and as such, hydrophilic (high polarity) components in the sample will elute prior to the more hydrophobic (lower polarity) compounds. A review of the structures of prazosin and prazosin metabolites (see Figure II-2) indicates that the metabolites would be more polar than the parent compound. It is reasonable to hypothesize that these time-dependent peaks are metabolites of prazosin, and the conditions of the isocratic assay may be such that the more polar metabolites elute together and very rapidly.

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[1] If these time-dependent peaks represent prazosin metabolites, each compound would have a different molecular weight and fluorescence activity. The peak height ratios and subsequent "relative concentrations" were determined from the prazosin standard curve so that a curve of "concentration" vs time could be constructed. The "relative concentration" is used only to provide an estimate of concentrations of the various components present in the tracing.

[2] C-18 μBondapak (30cm X 3.9mm ID, 10 μ particle size), Water Associates, Milford, MA

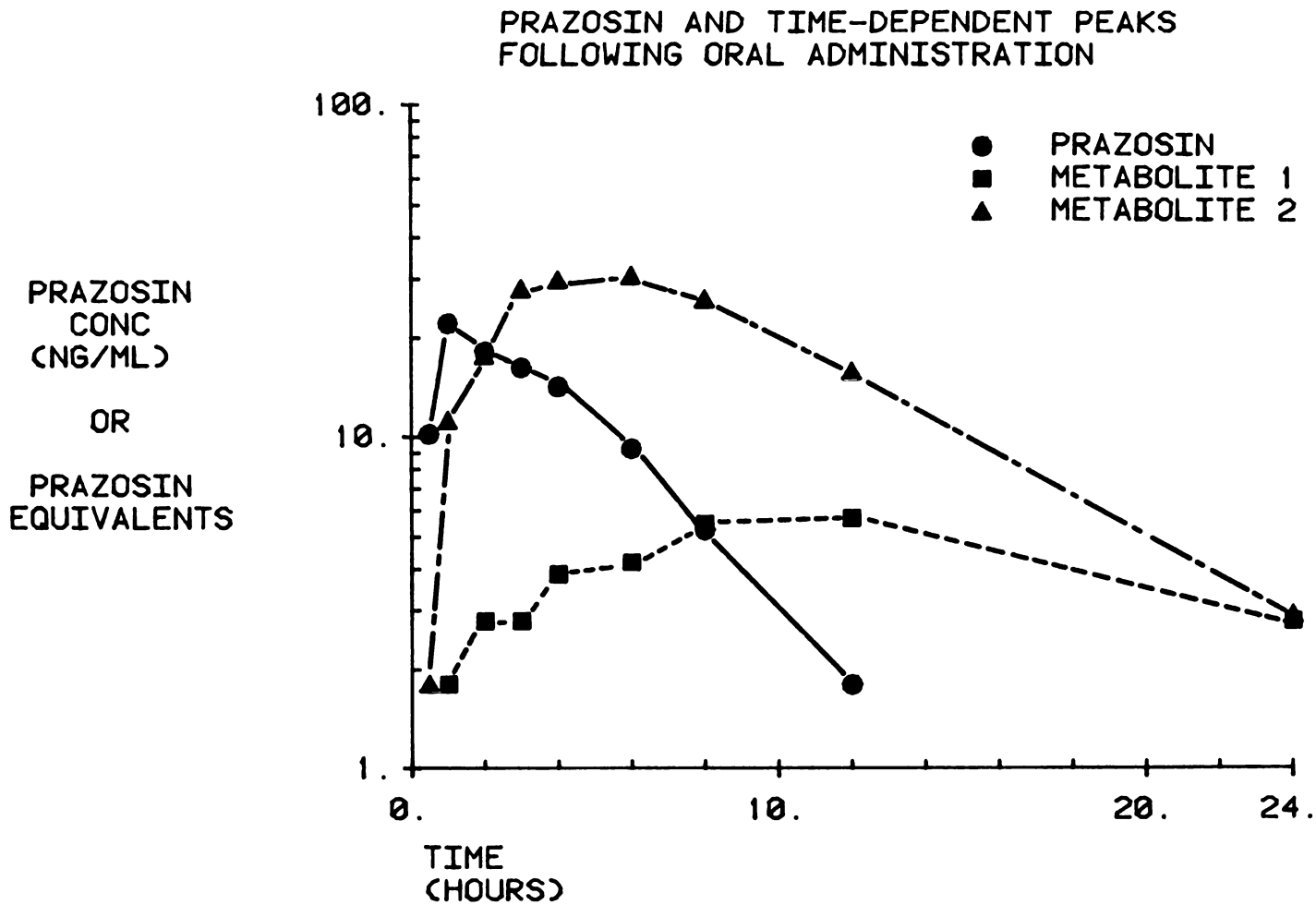

Figure VI-2. Plasma concentration-time curves for prazosin and two time-dependent peaks. Data was obtained by isocratic analysis of plasma from a normal subject following a 5 mg oral prazosin dose.

B. Specific Objectives

To develop an HPLC fluorescence gradient assay to further elucidate the number of time-dependent peaks found in the plasma or whole blood of humans, or animals, following prazosin administration.

C. Experimental

1. Reagents

Prazosin hydrochloride, carbamazepine, phosphoric acid, acetonitrile and distilled water were the same as described in Chapter II Section B.1. One known metabolite of prazosin, 2,4-diamino-6,7-dimethoxyquinazoline[3] (XVIII, Figure II-2), was obtained through the courtesy of Pfizer Central Research.[4]

2. Instrumentation

A Varian[5] Model 5000 Liquid Chromatograph equipped with

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[3]Pfizer compound: CP-10,215, lot#: 3442-55-1
[4]Pfizer Inc., Groton, CT
[5]Varian Associates, Palo Alto, CA
[6]Rheodyne Inc., Cotati, CA

a Rheodyne[6] loop injector, a Perkin-Elmer[7] fluorescence spectrophotometer (Model 650-10S) and a Linear[8] dual pen recorder were used with a C-18 reversed phase column[9] (25cm X 4.6cm ID, 10 μ particle size). The fluorescence detector was operated at an excitation wavelength of 340 nm, and an emission wavelength of 384 nm.

3. Preparation of stock solutions

Prazosin and carbamazepine stock solutions were prepared as described in Chapter III Section B.3. The known metabolite (3.175 mg) was dissolved in 10 ml of MeOH and the mixture sonicated for 10 minutes to ensure dissolution. The solution was then diluted with distilled water to a final volume of 50 ml in a graduated volumetric flask.

4. Gradient assay for prazosin and drug-related products in plasma and whole blood

Sample preparation for the gradient assay is identical to that described in Chapter III Section B.4 for the prazosin assay by either Method I or II. To reduce the possibility of interference with the fluorescence detection of pra-

[7]Perkin-Elmer Corp., Instrument Division, Norwalk, CT
[8]Linear Instrument Corp., Irvine, CA
[9]Alltech Associates, Deerfield, IL

zosin and prazosin metabolites, carbamazepine was used as the internal standard rather than the fluorescence internal standard tiodazosin.

The Varian Model 5000 is composed, in part, of three solvent reservoirs, proportioning valves and a solvent-delivery system. A pair of microprocessor-controlled proportioning valves admit solvent from two of the three reservoirs in an accurately metered ratio to the pump, which then delivers the solvent to the column. The gradient program is stored in the instrument memory, which contributes to the highly reproducible gradient solvent flow.

The gradient program and solvent composition currently in use were developed in an attempt to separate all components present in the sample. The solvent system was drawn from two reservoirs, one reservoir containing CH_3CN and the other an aqueous buffer of 0.1% H_3PO_4, pH 3.6. The solvent composition was programmed in the following 19 minute cycle: 15% CH_3CN for 3 minutes, increasing linearly to 35% over the next 10 minutes, and 35% CH_3CN for 6 minutes (see Figure VI-3). The flow rate throughout the assay was 2.0 ml/min.

D. Results and Discussion

Representative chromatograms of blank plasma and plasma spiked with prazosin and the known prazosin metabolite are shown in Figure VI-4. The retention times for prazosin and

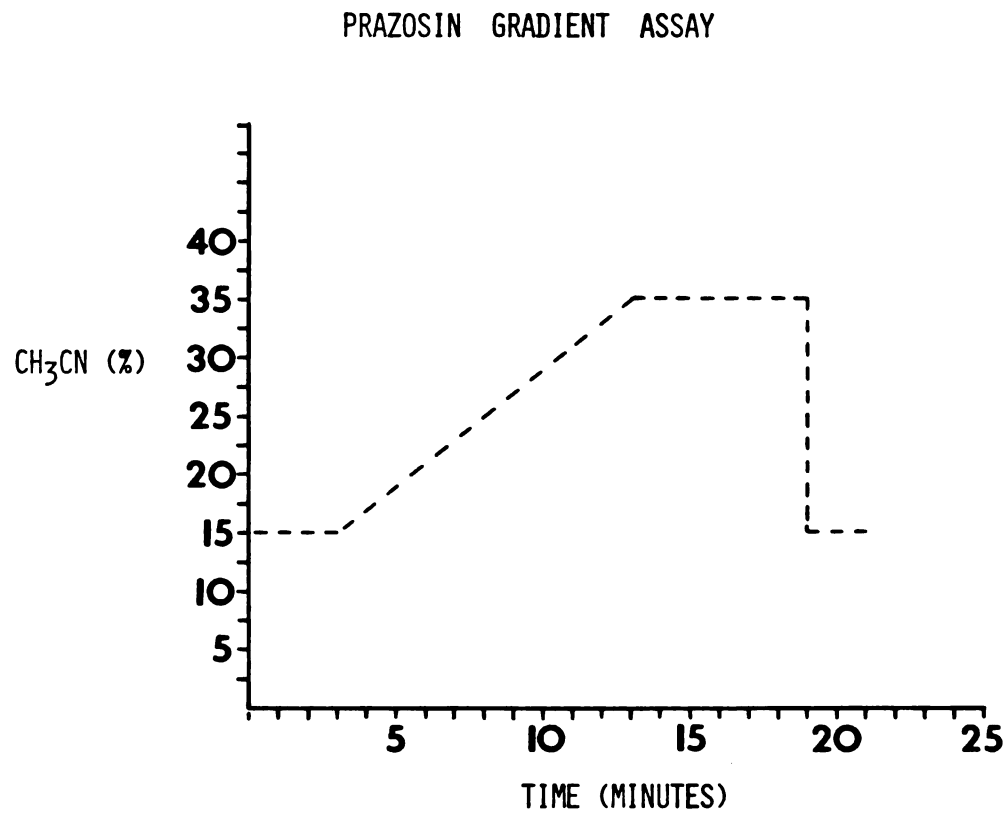


Figure VI-3. Solvent program for prazosin gradient assay.

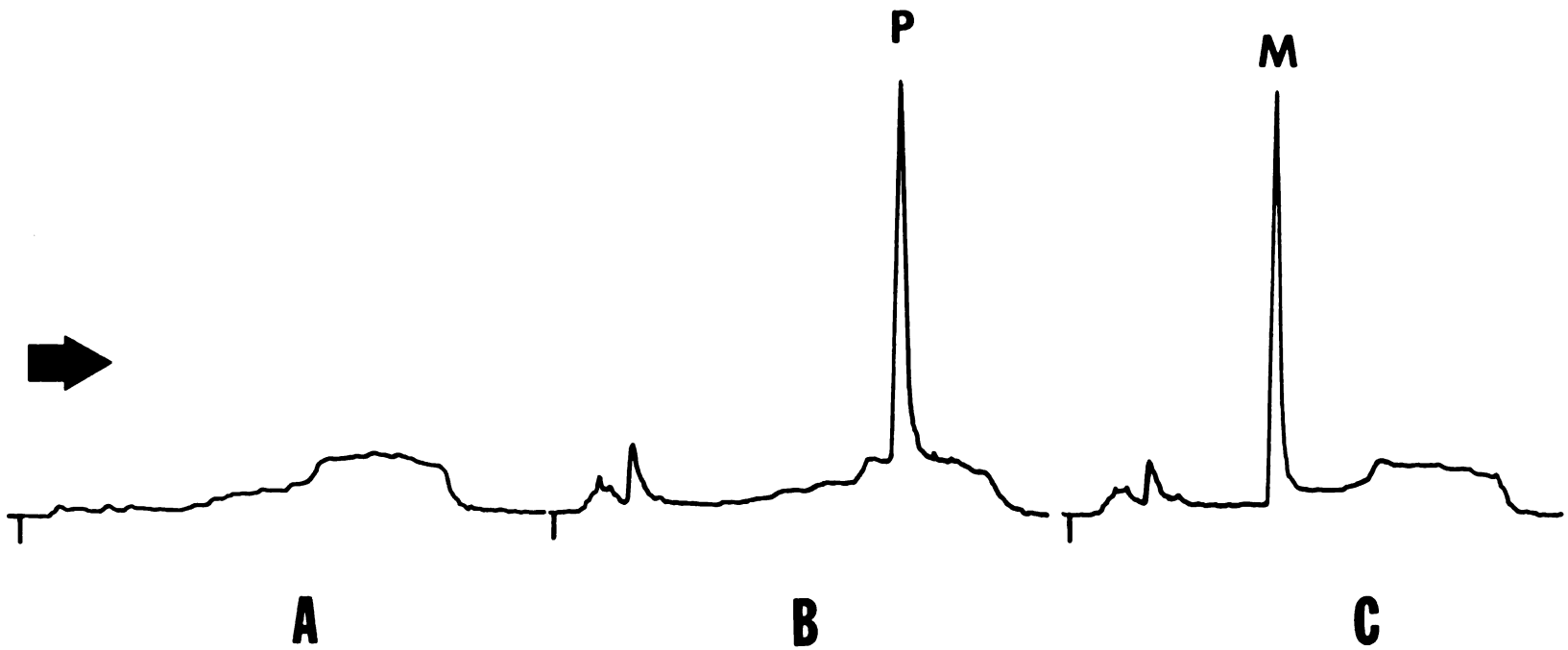
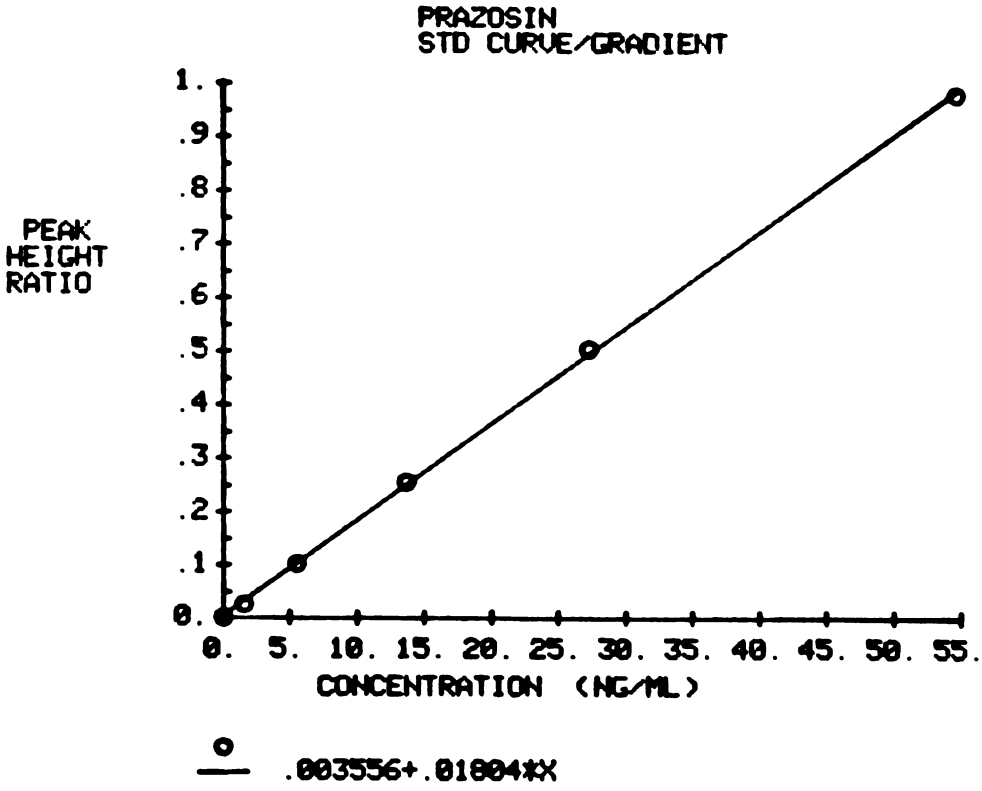


Figure VI-4. Chromatograms from gradient assay for plasma spiked with prazosin (P) and 2,4-diamino-6,7-dimethoxyquinazoline (M). (A) Blank plasma; (B) plasma spiked with prazosin standard, retention time of 16 minutes; and (C) plasma spiked with prazosin metabolite (M); retention time of 10 minutes.

the 2,4-diamino metabolite under the above conditions are 16 and 10 minutes, respectively. The limit of detection for prazosin and the metabolite utilizing this method (signal-to-noise ratio 4:1) is approximately 1 ng/ml.

Calibration graphs for prazosin were constructed over the concentration range of 1.6 to 164 ng/ml from fortified samples using the sample preparation procedure described above. Calibration curves for the known prazosin metabolite were prepared using the same technique as above by adding the compound to blank plasma in an amount that produced concentrations from 1.4 to 143 ng/ml. Calibration curves for prazosin and metabolite were prepared in separate plasma samples as well as for both compounds added to the same plasma sample. Prazosin and metabolite concentrations were determined by peak height ratios.

All calibration curves were linear over the concentration ranges tested. Figure VI-5 is the calibration graph using the gradient assay for samples fortified with prazosin alone. This standard curve was constructed over the concentration range of 1.6 to 54.7 ng/ml. The calibration curve for metabolite quantitation, Figure VI-6, is constructed from fortified plasma samples over the range of 1.4 to 47.6 ng/ml. Linear least squares regression equations and coefficients of determination for the prazosin and metabolite curves were: $Y = .018X + .004$, $r^2 = .999$ and $Y = .026X - .017$, $r^2 = .998$, respectively.



NUMBER OF DATA POINTS = 6
CORRELATION COEFFICIENT R = .9998265 R-SQUARED = .9996531
STANDARD DEVIATION OF REGRESSION = .0078759

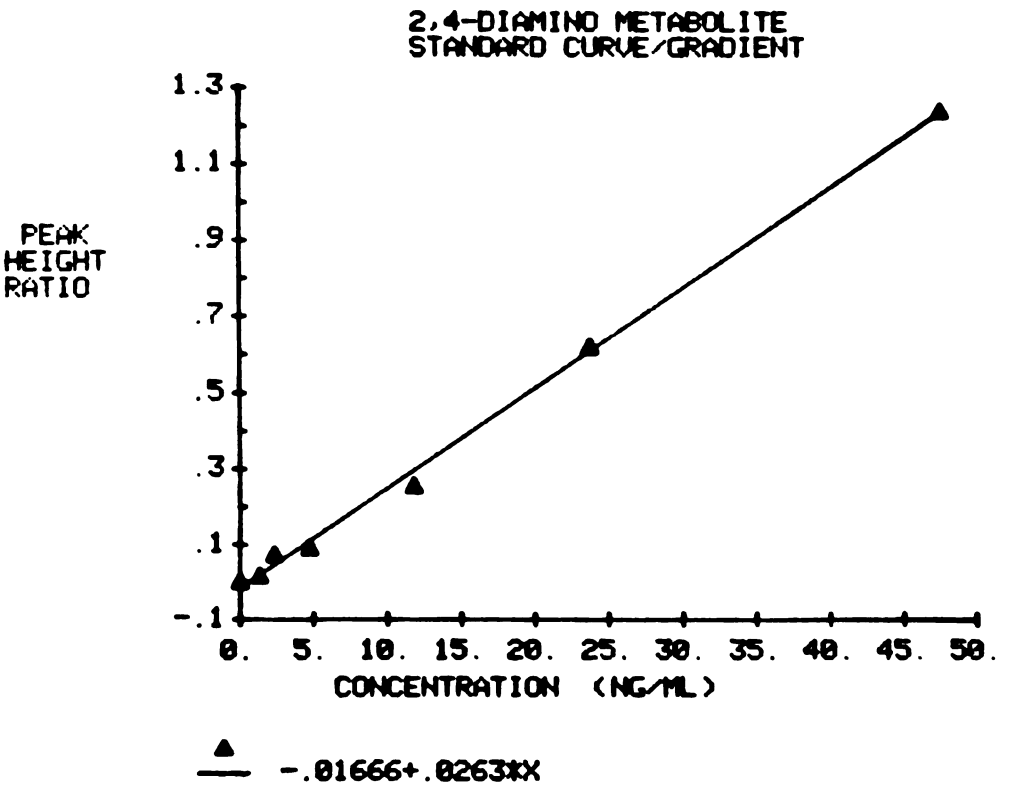
PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	.003556494	.004316016	.8240223	.456
SLOPE	.01804145	.0001680457	107.3604	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.7149712	1.	.7149712	11526.25	.0001
RESIDUAL	.0002481192	4.	$6.202981X10^{-5}$		

Figure VI-5. Prazosin standard curve for gradient assay with statistical evaluation.



NUMBER OF DATA POINTS = 7
CORRELATION COEFFICIENT R = .9987682 R-SQUARED = .9975379
STANDARD DEVIATION OF REGRESSION = .02477444

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	-.01666151	.01210444	-1.376479	.227
SLOPE	.02629631	.000584255	45.00827	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	1.243347	1.	1.243347	2025.744	.0001
RESIDUAL	.003068864	5.	.0006137729		

Figure VI-6. Standard curve for the gradient assay of the 2,4-diamino-metabolite of prazosin, with statistical evaluation.

When both compounds were added to the same plasma sample and then co-chromatographed with the gradient assay, excellent separation was obtained (see Figure VI-7). Calibration curves, constructed from 1.6 to 164 ng/ml for prazosin and from 1.4 to 143 ng/ml for the 2,4-diamino metabolite, resulted in the following linear least squares regression equations: $Y = .017X - .022$ ($r^2=.997$) and $Y = .028X - .039$ ($r^2=.997$), respectively (see Figure VI-8).

To determine if a drug-related peak with a retention time equal to that of the 2,4-diamino metabolite was generated in human subjects, standard curves from plasma samples fortified with both prazosin and the 2,4-diamino metabolite were prepared. These samples, along with plasma samples from a CHF patient and a normal volunteer, were then assayed using the gradient procedure. The 2,4-diamino metabolite was not found in the plasma of either the CHF patient or the normal volunteer following prazosin administration. However, it was observed that the plasma from the CHF patient generated more time-dependent peaks than was evident in the plasma from the normal volunteer. Subsequent analysis of plasma samples from other CHF patients and normal volunteers indicated that the number of drug-related peaks was routinely greater in the individuals with heart failure. Gradient analyses of samples (using carbamazepine as the internal standard, UV chromatograms not shown) from a congestive heart failure patient are shown in Figure VI-9.

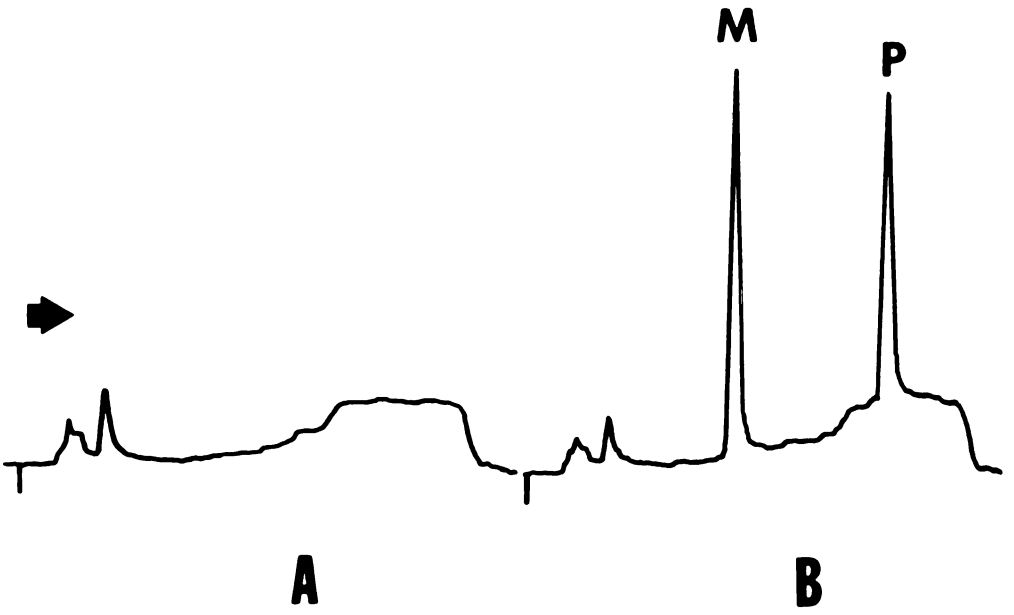


Figure VI-7. HPLC chromatograms from gradient assay of plasma samples spiked with 2,4-diamino-6,7-dimethoxyquinazoline (M) and prazosin (P). (A) blank plasma; (B) metabolite concentration of 143 ng/ml and prazosin concentration of 164 ng/ml.

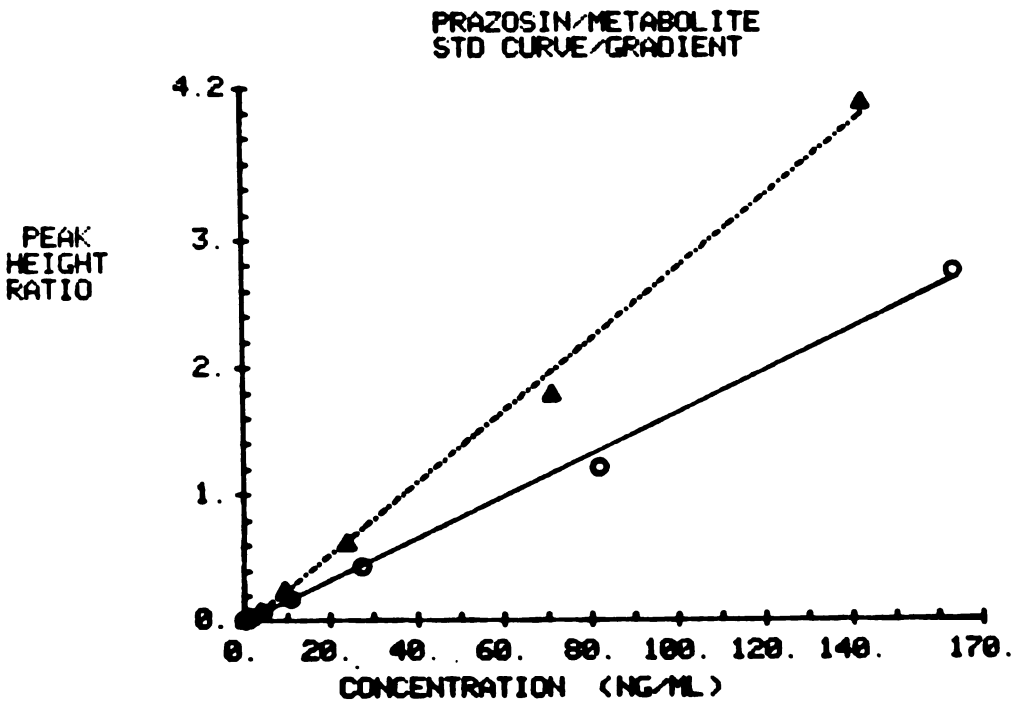
NUMBER OF DATA POINTS = 7
CORRELATION COEFFICIENT R = .9984894 R-SQUARED = .9969811
STANDARD DEVIATION OF REGRESSION = .06127083

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	-.02236544	.02869464	-.7794292	.471
SLOPE	.01660771	.0004086979	40.63567	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	6.19901	1.	6.19901	1651.257	.0001
RESIDUAL	.01877058	5.	.003754115		



NUMBER OF DATA POINTS = 7
CORRELATION COEFFICIENT R = .9984282 R-SQUARED = .9968588
STANDARD DEVIATION OF REGRESSION = .09269168

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	-.03890371	.0434035	-.8963266	.411
SLOPE	.02824961	.0007091808	39.83414	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	13.63303	1.	13.63303	1586.759	.0001
RESIDUAL	.04295874	5.	.008591747		

Figure VI-8. Standard curves for prazosin and metabolite when co-administered using the gradient assay, with statistical evaluation for prazosin (top) and metabolite (bottom).

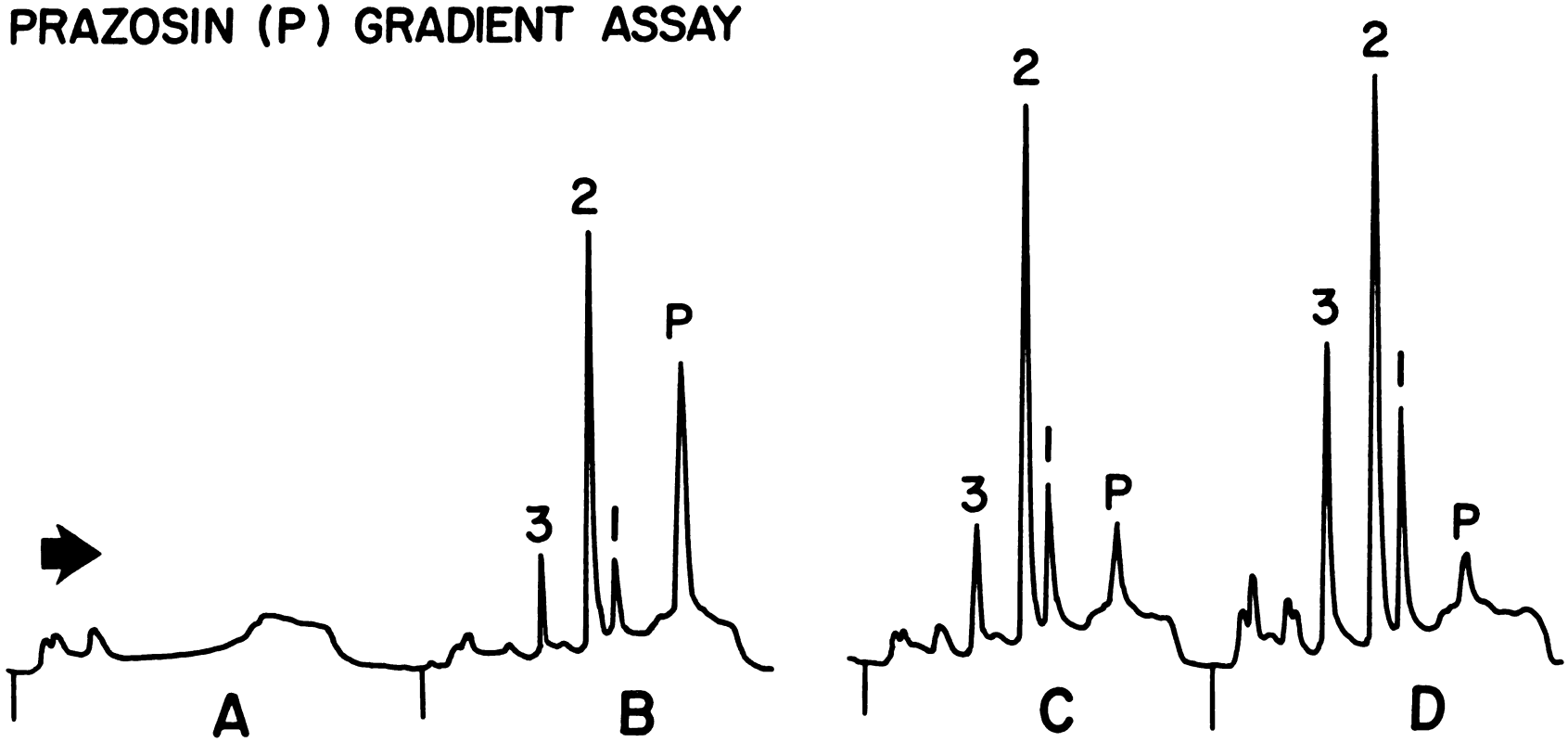


Figure VI-9. Chromatograms of plasma samples from a CHF patient using the HPLC gradient fluorescence assay (Method I, UV tracing of the internal standard not shown). (A) blank plasma; (B), (C) and (D) are chromatograms of plasma samples following oral prazosin administration; samples taken at 6, 12 and 24 hours post-dosing.

The samples presented were selected at specific times to show: 1) that the peaks of interest were generated after prazosin administration, and 2) that the heights of the unidentified peaks continued to increase long after the decline in unchanged prazosin has begun. These data, presented in Figure VI-10, indicate the time course of the three time-dependent peaks, in addition to the parent compound, observed in the plasma from a CHF patient. The apparent terminal half-lives of the additional time-dependent peaks were, in all cases, greater than the half-life of prazosin in each individual studied.

The importance of this chromatographic method lies in the fact that if these time-dependent peaks are metabolites of prazosin, this assay is the first to provide some physical evidence for their presence in plasma. Further, if these metabolites possess hypotensive activity (three of the metabolites have been implicated, Chapter II Section C) then by the nature of their longer half-lives in plasma and following multiple dosing of prazosin, these compounds will exhibit significant accumulation in the plasma and thus, may contribute significantly to the overall hypotensive effect.

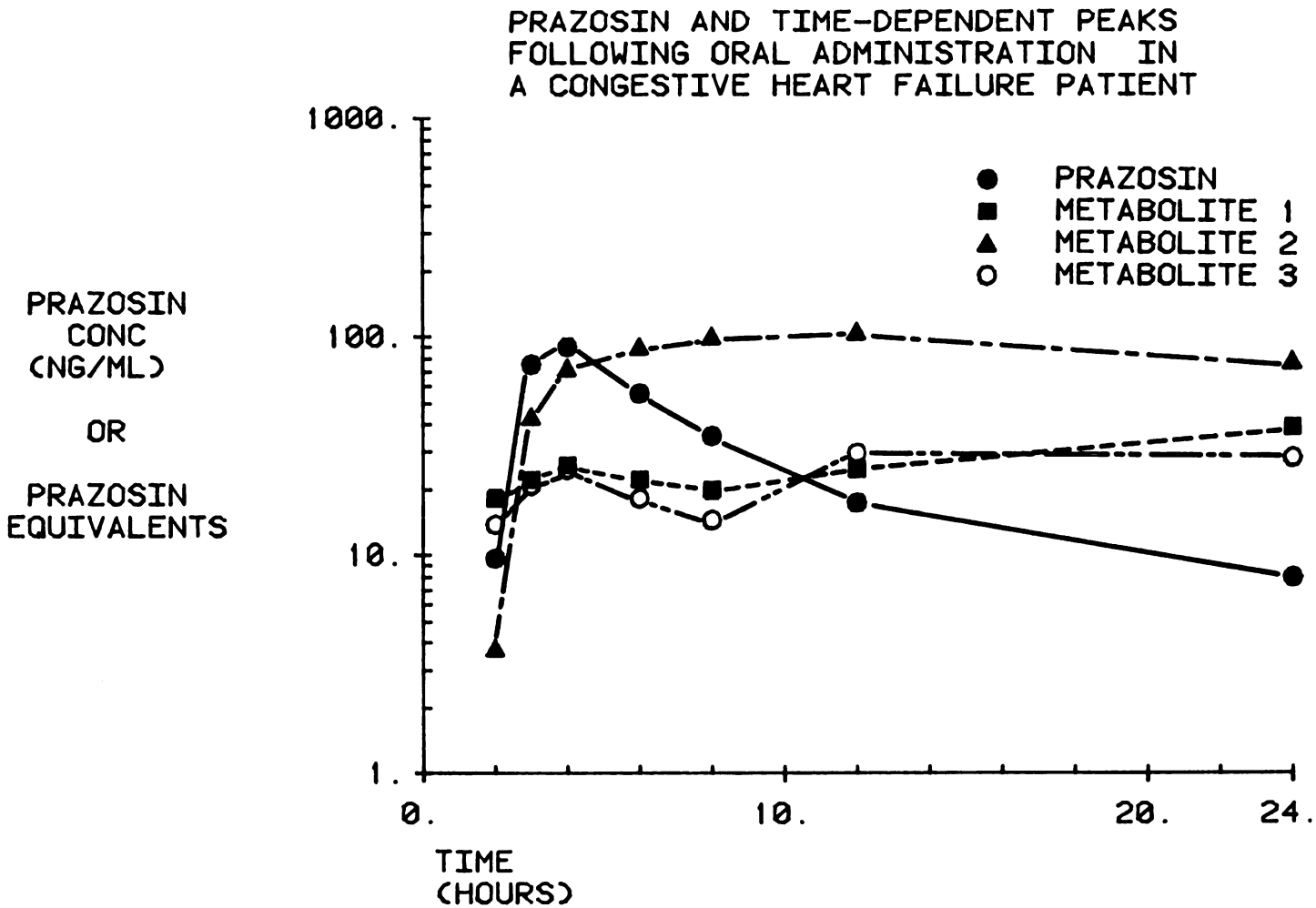


Figure VI-10. Plasma concentration-time curves of prazosin and three suspected metabolites from a CHF patient following the administration of a 5 mg oral prazosin capsule.

CHAPTER VII

THE EFFECT OF ACUTE AND CHRONIC CIMETIDINE ADMINISTRATION ON PRAZOSIN PHARMACOKINETICS IN NORMAL HUMAN SUBJECTS

A. Background

Prazosin decreases intra-arterial and venous pressure by vascular dilation secondary to alpha-adrenoceptor blockade. Following the initial reduction in blood pressure by prazosin, reflexogenic mechanisms would normally increase the heart rate and stroke volume, thus augmenting the cardiac output (see Figure I-5). In normal individuals, prazosin administration has not been shown to significantly alter the cardiac output, and subsequently, perfusion of the various organs is unaffected. In subjects who are blood flow compromised (e.g., heart failure and complicated hypertension) elevated pressures and/or decreased cardiac efficiency may lead to tissue congestion and reduced organ perfusion. If the blood flow to the liver is reduced, regardless of the mechanism, drug metabolism may be altered. It has been shown that the clearance of high extraction ratio compounds is determined by the blood flow to the eliminating organ. The removal of low extraction ratio compounds can also be affected, not by alterations in blood flow, but by a reduction in the ability of the clearing organ to function (e.g.,

hepatic congestion has been shown to reduce the clearance of low extraction ratio drugs that are cleared exclusively by the liver).

Cimetidine is an H_2-receptor antagonist used in the treatment of gastric and duodenal ulcers. Since 1979, when Serlin et al. (153) first reported the interaction between cimetidine and warfarin, there have been a considerable number of reported interactions between cimetidine and other drugs (154-160). These reports indicated that when coadministered with other compounds, cimetidine has been shown to decrease the hepatic elimination of highly extracted drugs (e.g., morphine, propranolol) (154,155) as well as those agents with a low hepatic extraction ratio (e.g., warfarin, antipyrine, diazepam, chlordiazepoxide, theophylline (153,156). The mechanism of these interactions are thought to be due to two separate components: 1) the imidazole ring of cimetidine (XIX, Figure VII-1) is believed to antagonize hepatic mixed function oxygenase metabolism (161-163), and 2) the H_2 blocking effects of cimetidine have been reported to decrease hepatic blood flow as determined indirectly by the indocyanine green (ICG) clearance method. Whether cimetidine decreases liver blood flow or alters the ICG hepatic extraction ratio is currently in question.

Experimental evidence presented in Chapter V demonstrated that following prazosin administration to congestive heart failure patients as compared to normal controls, the

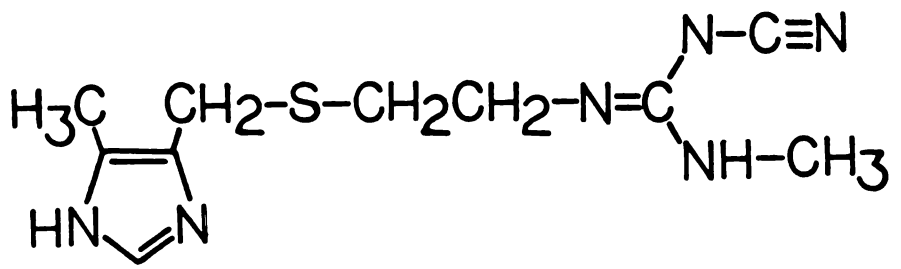


Figure VII-1. The structure of the H_2-receptor blocking agent, cimetidine (XIX).

AUC and half-life were increased and the CL/F was decreased significantly. Although an iv formulation is not available to directly measure the effect of heart failure on prazosin clearance, the data indicates that a reduction in the intrinsic clearance could account for all of the observed parameter changes. Because cimetidine has been shown to decrease hepatic metabolism (intrinsic hepatic clearance) and the probability exists for concomitant administration with prazosin, it is of considerable therapeutic importance to determine if cimetidine co-administration alters the disposition of prazosin.

B. Specific Objective

To determine if the disposition of prazosin is altered by concomitant cimetidine administration, prazosin pharmacokinetic parameters were determined following oral dosing in six healthy volunteers and after acute and chronic cimetidine administration, and 18 hours after cimetidine was discontinued.

C. Experimental

1. Subject selection

Six healthy subjects (3 male and 3 female) participated in this experiment. The experimental protocol was approved by the Committee on Human Research of the University of California, San Francisco and signed informed consent was given by all subjects prior to participation in the study. All subjects were within 20% of ideal body weight[1] and were normal upon physical examination. Laboratory values[2] and blood pressure were normal in all subjects. The subjects did not smoke, have a history of drug or alcohol abuse and had not taken agents known to induce hepatic metabolism (e.g., carbamazepine, phenobarbital, rifampin) or inhibit metabolism (e.g., INH, cimetidine, oral contraceptives) within six months preceding the study period.

2. Study design

The length of the complete protocol was 22 days, which included four study days of serial blood sampling following

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[1]Metropolitan Life Insurance Co., ideal weight based on sex, height, and frame

[2]CBC with differential, SMA-12 (including total protein, albumin, BUN, LDH, Alk. Phos., SGPT, SGOT), SMA-6 (serum electrolytes), α_1-acid glycoprotein, bilirubin and urinalysis

oral prazosin administration. Study day 1: Subjects fasted for 10 hours prior to drug administration, and at 7:30 AM on the morning of the study an indwelling catheter[3] was inserted in an antecubital vein. The catheter was kept patent by flushing the infusion set with a solution of heparinized normal saline following each blood sample. The subjects were kept in a resting supine position prior to the start of the study procedure and for 4 hours following prazosin administration. At 8:00 AM the experimental protocol was started. Subjects were administered a single 2 mg oral capsule of prazosin[4] (lot #: ASC 1/10/81B6 515C) with 300 ml apple juice.

Blood samples were drawn into 12 ml Monoject^{R} single use syringes[5] and immediately tranferred to Venoject^{R} blood collection tubes containing 143 IU of lithium heparin.[6] Blood was sampled for analysis prior to prazosin dosing (blank) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours following the oral dose. The samples were centrifuged immediately after collection for 10 minutes at 1500Xg and the plasma was separated from packed cells, placed in two dram screw cap vials[7] and stored at -20°C. Additional blood samples were obtained to determine the plasma to whole blood

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[3]Butterfly^{R} INT 21G X 3 1/2" tubing, Abbott Hospital Inc., North Chicago, IL

[4]Minipress, Pfizer Inc.

[5]Sherwood Medical Industries, Deland, FL

[6]Terumo Medical Corp., Elkton, MD

[7]Kimble Products Division, Owens-Illinois, Toledo, OH

concentration ratio, and were stored with the plasma samples.

Lunch and dinner were provided at 4 1/2 and 8 hours, respectively, following prazosin dosing. Meals of similar composition were provided on each of the four study days.

Study day 2: After a washout interval of at least two days, a 300 mg oral cimetidine[8] tablet (lot #: 700 D-lot 5651T13) and a 2 mg oral prazosin capsule were administered together, again with apple juice. Following the 24 hour prazosin sample, chronic cimetidine administration was initiated. Each subject took 300 mg oral cimetidine four times daily for eleven days. Study day 3: On the morning of the the third study day, the subjects returned with their cimetidine prescriptions. Tablets were counted for certification of compliance to the experimental protocol. At 8:00 AM prazosin (2 mg capsule) and cimetidine (300 mg tablet) were administered concurrently. As performed on previous study days, blood was sampled serially for prazosin analysis. After the last prazosin sample was taken, chronic cimetidine dosing was continued for five additional doses. This was done to ensure that prazosin and possible prazosin metabolites be completely cleared from the subject. To determine if there was a residual effect following cimetidine dosing on prazosin disposition, approximately 18 hours prior to the last study day cimetidine was

[8]Tagamet, Smith Kline & French

discontinued. Study day 4: Prazosin was administered as a 2 mg oral capsule, and blood was sampled to 24 hours. Approximately two days following the last study day, all subjects returned for routine blood and urine laboratory analysis.

3. Prazosin assay procedure

Plasma samples collected for prazosin assay were analyzed using the isocratic procedure of Method II described in Chapter III. Calibration graphs were constructed by fortifying pooled plasma samples over the concentration range of 1.0 to 24.0 ng/ml. Plasma concentrations were determined from peak height ratios of prazosin to internal standard. The mean slope of five calibration curves was 0.099399 with a coefficient of variation of 2.38%. The mean coefficient of determination for these five graphs was 0.9996 $\pm$ 0.0003.

4. Calculations

Prazosin pharmacokinetic parameters were calculated using non-compartmental methods as described in Chapter V, Section C.4. Statistical analyses of the data were performed using one-way analysis of variance, and repeated using the Newman-Keuls multiple comparison procedure.

D. Results

Prazosin pharmacokinetic parameters were determined for each subject on each of four study days following the administration of a 2 mg oral capsule. Plasma concentration-time curves for each study day in two subjects (MS and KP) are shown in Figures VII-2 and VII-3. These curves were selected to show the intra-subject variability in the peak plasma concentration and areas under the plasma concentration-time curves, although a comparison of the mean data from all subjects was very similar regardless of cimetidine administration (see Tables VII-1 and VII-2). The time of the peak concentration (Table VII-3), the rate constant of elimination (Table VII-4), plasma half-life (Table VII-5) and the blood clearance divided by the availability (CL_B/F) (Table VII-6) are also unchanged by either acute or chronic cimetidine dosing.

Each data table was analyzed for the probability of a statistically significant difference using the analysis of variance. No statistical difference was observed in the pharmacokinetics of prazosin when administered alone, with acute or chronic cimetidine dosing, or 18 hours after the discontinuation of chronic cimetidine administration.

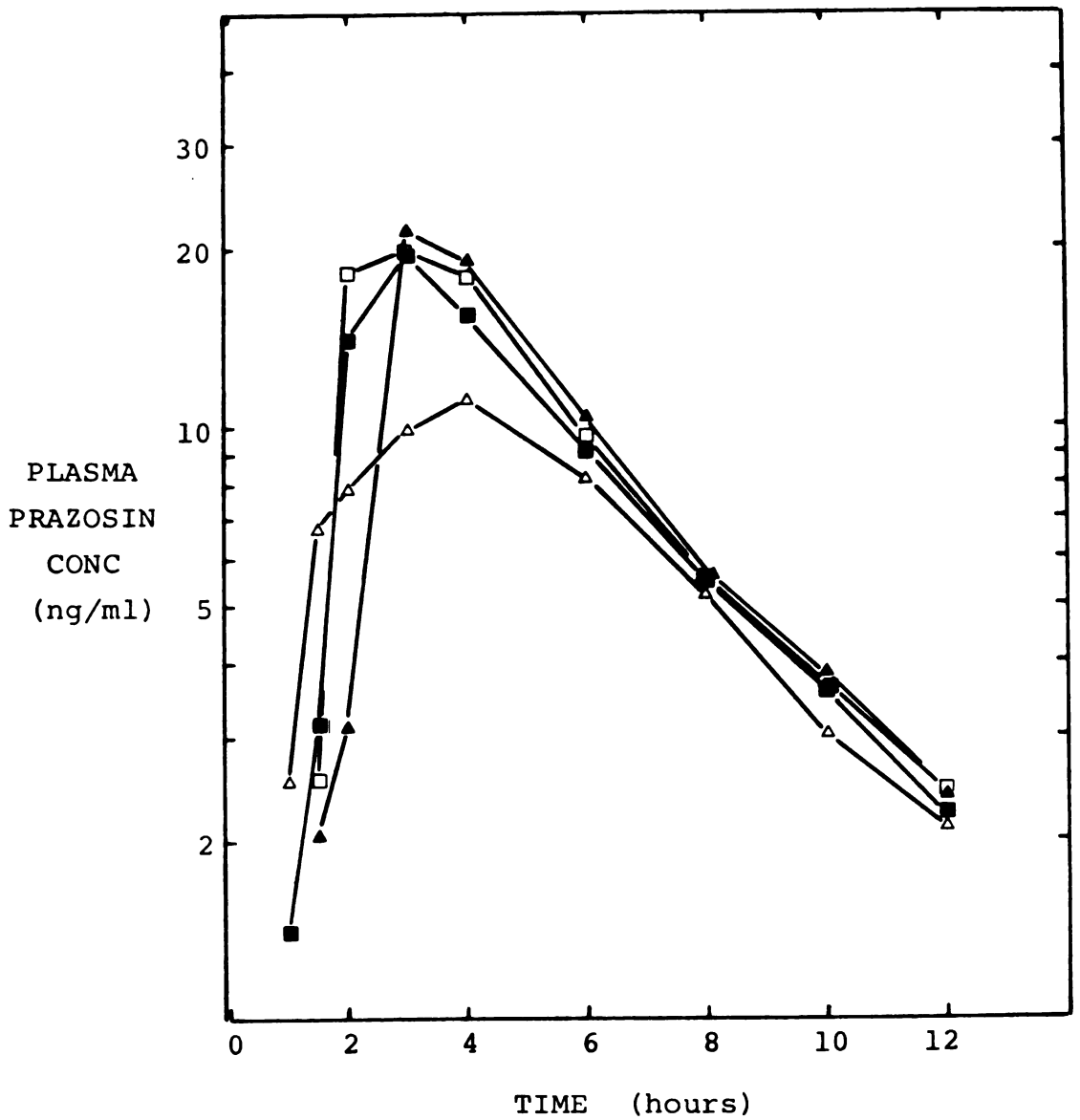

Figure VII-2. Plasma concentration-time curves from subject MS following 2 mg oral prazosin on four separate study days [study day 1 (■), study day 2 (□), study day 3 (▲) and study day 4 (△)].

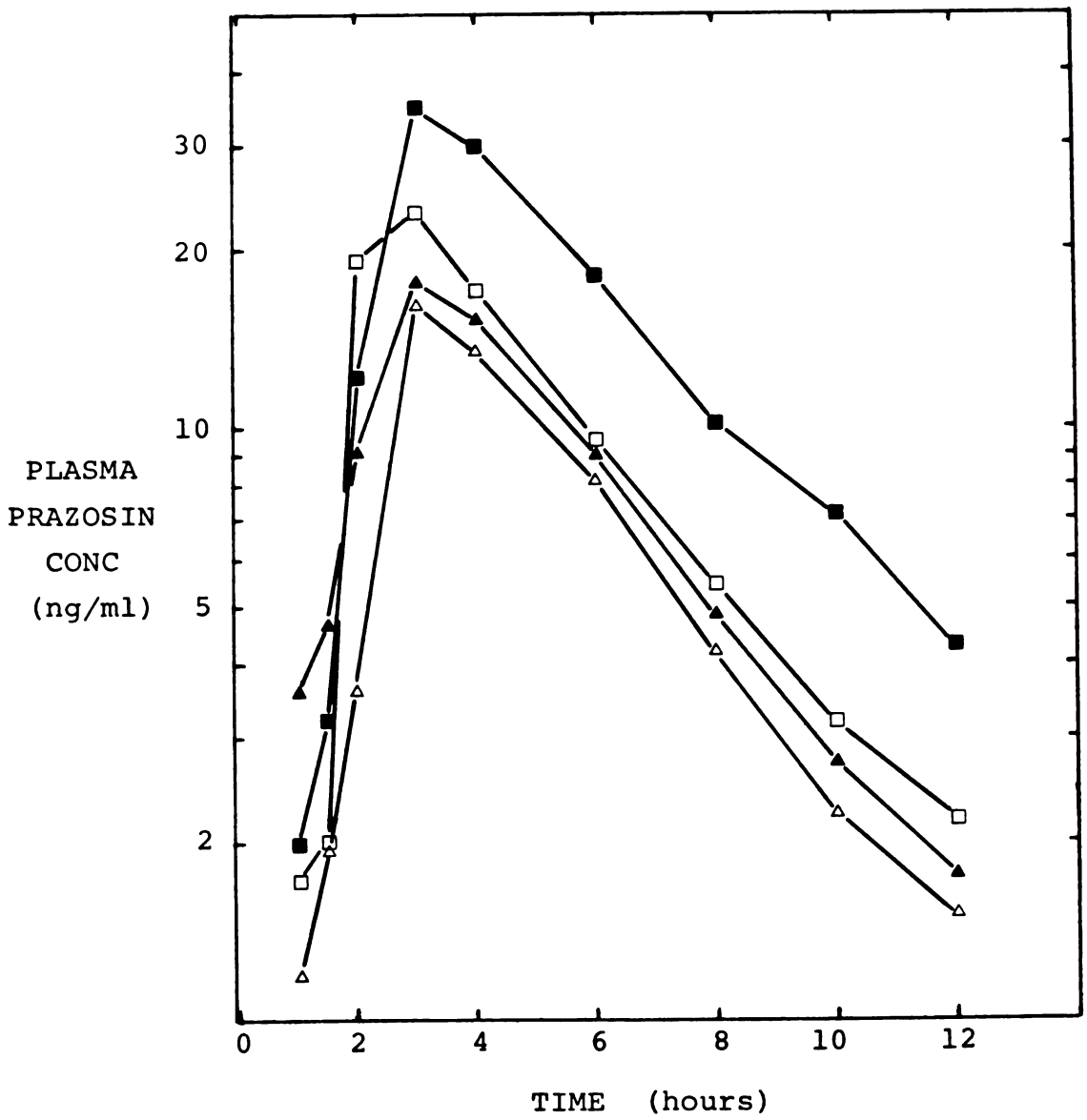

Figure VII-3. Plasma concentration-time curves from subject KP following 2 mg oral prazosin on four separate study days [study day 1 (■), study day 2 (□), study day 3 (▲) and study day 4 (△)].

Table VII-1. Peak prazosin plasma concentration [C_{peak}, (ng/ml)] observed in each subject following a 2 mg oral dose on four separate study days.

SUBJECT	STUDY DAY 1	STUDY DAY 2	STUDY DAY 3	STUDY DAY 4
AL	27.4	13.0	21.3	13.8
JW	26.9	18.7	13.1	20.3
KP	34.9	23.2	17.6	16.1
MS	19.8	19.7	21.5	11.2
SS	10.2	20.5	20.6	18.0
MG	13.8	21.2	19.0	18.4
MEAN	22.2	19.4	18.9	16.3
± SD	9.3	3.5	3.2	3.3

Table VII-2. Area under the plasma concentration-time curve [AUC_P (μg·hr/ml)] in each subject following a 2 mg oral prazosin dose on four separate study days.

SUBJECT	STUDY DAY 1	STUDY DAY 2	STUDY DAY 3	STUDY DAY 4
AL	123.9	81.2	105.4	66.7
JW	104.4	97.6	75.1	91.9
KP	185.4	112.0	92.5	76.0
MS	103.8	113.0	104.4	80.5
SS	55.8	98.7	114.4	87.9
MG	64.6	96.8	88.8	87.3
MEAN	106.3	99.8	96.7	81.7
± SD	46.6	11.6	14.1	9.3

Table VII-3. Time of peak prazosin concentration [T_{peak}, (hrs)] in each subject following a 2 mg oral dose on four separate study days.

SUBJECT	STUDY DAY 1	STUDY DAY 2	STUDY DAY 3	STUDY DAY 4
AL	3	6	3	3
JW	3	4	4	3
KP	3	3	3	3
MS	3	3	3	4
SS	4	3	3	3
MG	3	2	1	1
MEAN	3.2	3.5	2.8	2.8
± SD	0.4	1.4	1.0	1.0

Table VII-4. The rate constant of elimination [λ_z,(hr^{-1})] determined in each subject following a 2 mg oral dose on four separate study days.

SUBJECT	STUDY DAY 1	STUDY DAY 2	STUDY DAY 3	STUDY DAY 4
AL	0.238	0.279	0.321	0.296
JW	0.285	0.299	0.239	0.267
KP	0.245	0.259	0.291	0.309
MS	0.242	0.238	0.256	0.240
SS	0.222	0.294	0.288	0.288
MG	0.281	0.305	0.290	0.273
MEAN	0.252	0.279	0.281	0.279
± SD	0.025	0.026	0.029	0.024

Table VII-5. Prazosin plasma half-life [$t_{\frac{1}{2}}$ (hrs)] in each subject following a 2 mg oral dose on four separate study days.

SUBJECT	STUDY DAY 1	STUDY DAY 2	STUDY DAY 3	STUDY DAY 4
AL	2.91	2.48	2.16	2.34
JW	2.43	2.32	2.90	2.60
KP	2.83	2.68	2.38	2.25
MS	2.86	2.91	2.71	2.88
SS	3.13	2.36	2.41	2.57
MG	2.46	2.27	2.39	2.54
MEAN	2.77	2.50	2.49	2.53
± SD	0.27	0.25	0.27	0.22

Table VII-6. Blood clearance divided by the availability [CL_B/F (L/hr)] determined in each subject following a 2 mg oral dose on four separate study days.

SUBJECT	STUDY DAY 1	STUDY DAY 2	STUDY DAY 3	STUDY DAY 4
AL	21.6	33.0	25.4	40.2
JW	25.7	27.5	35.7	29.2
KP	14.5	24.0	29.0	35.3
MS	25.8	23.8	25.7	33.3
SS	48.1	27.2	23.4	30.5
MG	41.5	27.7	30.2	30.7
MEAN	29.5	27.2	28.2	33.2
± SD	12.7	3.3	4.4	4.1

E. Discussion

The effect of cimetidine co-administration on the disposition of various compounds is currently of interest following numerous reports of a reduction in the clearance of the concomitantly administered compounds. Experimental evidence indicates that cimetidine decreases the clearance of hepatically cleared compounds by two different mechanisms: 1) by reducing liver blood flow; and, 2) by binding to the heme moiety of cytochrome P-450 and P-448, thus inhibiting the hepatic drug-metabolizing activity of the mixed function oxygenase enzymes (MFOE) system. This two-fold effect has been shown to alter the clearance of both high and low hepatic extraction ratio compounds.

The blood flow to the liver is the principal determinant of clearance for high hepatic extraction compounds (e.g., lidocaine, morphine, propranolol). Cimetidine appears to decrease the clearance of these drugs by reducing liver blood flow. By inhibiting the MFOE system, there is a reduction in the clearance of low extraction ratio compounds that are dependent on the intrinsic metabolizing activity of the liver (e.g., warfarin, benzodiazepines, theophylline, β-blockers, anti-convulsants).

Prazosin is a moderate hepatic extraction ratio compound (Table II-1), assuming that following oral administra-

tion all of the compound is presented to the liver.[9] The clearance of prazosin would then appear to be influenced by both the hepatic blood flow and the intrinsic metabolizing activity, rather than either one alone.

Pharmacokinetic parameters were determined for prazosin following the administration to six healthy young adults of a 2 mg oral capsule only, concomitantly with either a single cimetidine oral dose or following eleven days of chronic cimetidine dosing, and 18 hours after the discontinuation of chronic cimetidine. The pharmacokinetic data from this investigation indicated that cimetidine co-administration, either acute or chronic, does not significantly affect prazosin disposition (Table VII-7).

An explanation for cimetidine's apparent lack of effect on prazosin pharmacokinetics may be simple or quite involved. Cimetidine may have no effect on prazosin kinetics and the results presented here would need no further explanation. There may be an effect of cimetidine on prazosin disposition but it may be too small to be seen among the characteristically high variability in the prazosin data. For example, if the reduction in liver blood flow was approximately 20% of normal [Feely et al. (155) reported 20 and 35% reductions in hepatic blood flow using ICG and

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[9] If this is not the case, and some drug is lost due to poor dissolution or gi metabolism, the extraction ratio would be smaller than the 40 to 50% predicted by the available bioavailability data.

Table VII-7. Mean prazosin pharmacokinetic parameters calculated on four study days. Each value is a mean of 6 observations (SD in parentheses).[a]

STUDY DAY	C_{peak} (μg/L)	T_{peak} (hrs)	λ_z (hrs^{-1})	$t_{½z}$ (hrs)	$AUC_{0_P}^{\infty}$ (μg·hr/ml)	CL_B/F (L/hr)
1[b]	22.2	3.2	0.252	2.77	106.3	29.5
	(9.3)	(0.4)	(0.025)	(0.27)	(46.6)	(12.7)
2[c]	19.4	3.5	0.279	2.50	99.8	27.2
	(3.5)	(1.4)	(0.026)	(0.25)	(11.6)	(3.3)
3[d]	18.9	2.8	0.281	2.49	96.7	28.2
	(3.2)	(1.0)	(0.029)	(0.27)	(14.1)	(4.4)
4[e]	16.3	2.8	0.279	2.53	81.7	33.2
	(3.3)	(1.0)	(0.024)	(0.22)	(9.3)	(4.1)

[a] No difference statistically (ANOVA) for any parameter

[b] 2 mg oral capsule

[c] 2 mg oral capsule administered with 300 mg oral tablet of cimetidine

[d] 2 mg oral capsule administered with 300 mg oral tablet of cimetidine during chronic cimetidine dosing

[e] 2 mg oral capsule administered 17 hrs after discontinuing cimetidine chronic dosing

propranolol clearances, respectively] and if the intrinsic metabolizing activity was reduced by 20% (163), then the following relationship

$$CL_T = \frac{Q_H \cdot \alpha \cdot CL_{int}}{Q_H + \alpha \cdot CL_{int}} \qquad \text{(VII-1)}$$

where α is the free fraction of prazosin in the plasma, would predict a 20% decrease in total hepatic clearance. The 20% reduction in clearance would be lost in the relatively large intrasubject variability (coefficient of variation: 11 - 42%, Table VII-7) observed for AUC_P and CL_B/F over the four study days. Finally, the effect on the blood flow and intrinsic metabolic activity by cimetidine may be negated by the pharmacological effects of prazosin. The complex effects of concomitant administration of an H_2-receptor blocker and an α_1-adrenoceptor blocking agent on hepatic, gastric, mesenteric and splanchnic blood flows have not been investigated.

In any event, this experiment has shown that acute or chronic cimetidine administration does not alter prazosin disposition. Clinically, concerns over the potential for an increased first-dose effect, as well as other adverse reactions, when prazosin is added to cimetidine therapy, or when cimetidine treatment is initiated in an individual taking chronic prazosin, appears to be unwarranted at this time.

SUMMARY AND CONCLUSIONS

Prazosin, an orally active selective $alpha_1$-adrenoceptor antagonist, is a potent vasodilating agent that is valuable in the treatment of hypertension and congestive heart failure. Animal studies have shown that prazosin undergoes extensive hepatic metabolism (>90%). Pharmacokinetic characterization following prazosin administration is incomplete as an intravenous dosage form for human use is unavailable. Analytical techniques for the analysis of prazosin in biological fluids are time consuming and require 2 to 4 ml of sample.

A direct-injection HPLC fluorescence assay was developed that is simple, rapid, sensitive, involves no extraction steps and requires only 0.2 ml of biological sample. Standard curves were highly reproducible and linear over a wide range of prazosin concentrations.

The bioavailability and disposition of prazosin (1 mg/kg) was studied in four beagle dogs following intravenous and oral administration. The experimentally determined bioavailability (73%) was in good agreement with that predicted (77%). Further studies in dogs revealed that when lower oral doses (1 and 5 mg) were administered, the experimental bioavailability was three-fold less than what was predicted. This data indicates the prazosin exhibits a dose-dependent bioavailability in dogs.

Prazosin pharmacokinetics after oral dosing was evaluated in 9 individuals in heart failure and 5 healthy controls. As compared to the healthy controls, the congestive heart failure patients exhibited statistically significant decreases in the prazosin blood clearance divided by availability and the rate constant for elimination and a statistically significant increase in the area under the plasma concentration-time curve.

Time-dependent peaks were observed during chromatographic analysis of the plasma and whole blood samples from heart failure and normal subjects. An HPLC fluorescence gradient assay was developed to elucidate all components present in the biological sample. Calibration curves for prazosin and the 2,4-diamino metabolite using this method were linear over all concentration ranges tested. It was observed that the number of drug-related peaks in samples from individuals with congestive heart failure were routinely greater than in samples from normal controls.

The effect of acute and chronic cimetidine therapy on prazosin pharmacokinetics was studied in 6 healthy young adults. A large intra- and inter-subject variability was apparent over the course of the study. No statistical difference was found when comparing the pharmacokinetic parameters of prazosin when administered alone, with acute or chronic cimetidine dosing, or 18 hours after the discontinuation of chronic cimetidine administration.

APPENDIX

THE BIOAVAILABILITY OF TIODAZOSIN, A COMPOUND STRUCTURALLY AND PHARMACOLOGICALLY RELATED TO PRAZOSIN

A. Experimental

1. Specific objective

Tiodazosin, 4-amino-6,7-dimethoxy-2-[4-(5-methylthio-1,3,4-oxadiazole-2-carbonyl)-piperazin-1-yl] quinazoline levulinate[1] is a new member of the aminoquinazoline class of alpha-blockers, of which prazosin was the prototype. Tiodazosin is currently being studied for its antihypertensive properties with comparison to the structurally similar compound prazosin (165-169). Generally, one would predict correlative pharmacologic and pharmacokinetic profiles after considering the structures of these two agents. However, significant differences do exist. Tiodazosin has been found to be a potent alpha-blocker, but its affinity for the post-synaptic alpha-receptor is 17 times less that that of prazosin (170). The extreme first-dose effect found with prazosin in human volunteers (97) has not been reported in human clinical trials with tiodazosin; and in these trials, the tiodazosin terminal half-life is reported to be 3 to 5

[1]BL5111R, Bristol Laboratories, Syracuse, NY

times greater than that of prazosin (168,169).

These differences, together with the variability in the bioavailability of the prototype prazosin, has led to interest in the bioavailability of this compound.

The objective of this experiment was to determine the bioavailability and disposition parameters of tiodazosin in male beagle dogs, with a comparison to prazosin pharmacokinetics at a similar dose.

2. Study design

Five male beagle dogs from 3 to 5 years of age and 11.2 to 15.2 kg body weight were studied. The animals were fasted for 18 hours prior to receiving the intravenous and oral doses, but water was always available. The parenteral solutions of tiodazosin (lot #: 77F655) were prepared by dissolving the compound (1 mg/kg free base equivalent) in water for injection, with some brief sonication to insure complete solubility. The parenteral solution was infused over 30 minutes. Blood samples were taken at 0, 10, 20, 30 (end of infusion), 40, 50, 60, 75, 90, 105, 120, 150, 180, 240, 360, 480, 600, 720, 900 and 1440 minutes. After a 7-day interval, tiodazosin (1 mg/kg free base equivalent) was dissolved in 75 ml water for injection and administered as an oral solution through a stomach tube. This was followed by washing the tube with an additional 25 ml of water for

injection, and removing the stomach tube immediately. A tiodazosin test solution was assayed by Method II and the concentration was shown not to be altered by passage through the stomach tube. Venous blood was then sampled at 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, 480, 600, 720, 900 and 1440 minutes. Additional whole blood samples were taken at various times, so as to include a wide concentration range, in both iv and po studies for the determination of the whole blood concentration and the subsequent plasma to whole blood concentation ratio.

All other experimental conditions, including animal handling and the disposition of samples, were the same as those described in Chapter IV, Section B.1.b. Routine laboratory tests were within normal limits preceding and following the testing periods.

3. Assay procedure

Tiodazosin concentrations in plasma and whole blood were determined as described in Chapter III Section C.2. Calibration curves were constructed over the concentration range of 14.5 to 869 ng/ml (see Figure III-9).

4. Calculations

All pharmacokinetic parameters were determined using non-compartmental methods, as discussed in Chapter IV Section B.1.d. Linear regression analysis was performed by the method of least squares, and the paired t-test was used in comparing iv and po half-lives following tiodazosin administration. Statistical analysis comparing prazosin and tiodazosin parameters was performed with the two-sample t-test.

B. Results

Tiodazosin levulinate was administered intravenously and orally in a dose of 1 mg/kg free base equivalent to five male beagle dogs. A representative plasma concentration-time profile from one animal is seen in Figure A-1. Information on the animals used in our study, the tiodazosin pharmacokinetic parameters for each animal, and the sample means and standard deviations are found in Table A-1. The terminal half-lives following the different routes of administration were not statistically significantly different. The mean values for the blood clearance and the steady-state volume of distribution were 6.75 $\pm$ 1.28 L/hr and 22.1 $\pm$ 8.2 liters, respectively. The mean experimental bioavailability for the oral solution calculated from Equation IV-1 was 26 $\pm$ 8%. However, using Equation IV-3, the

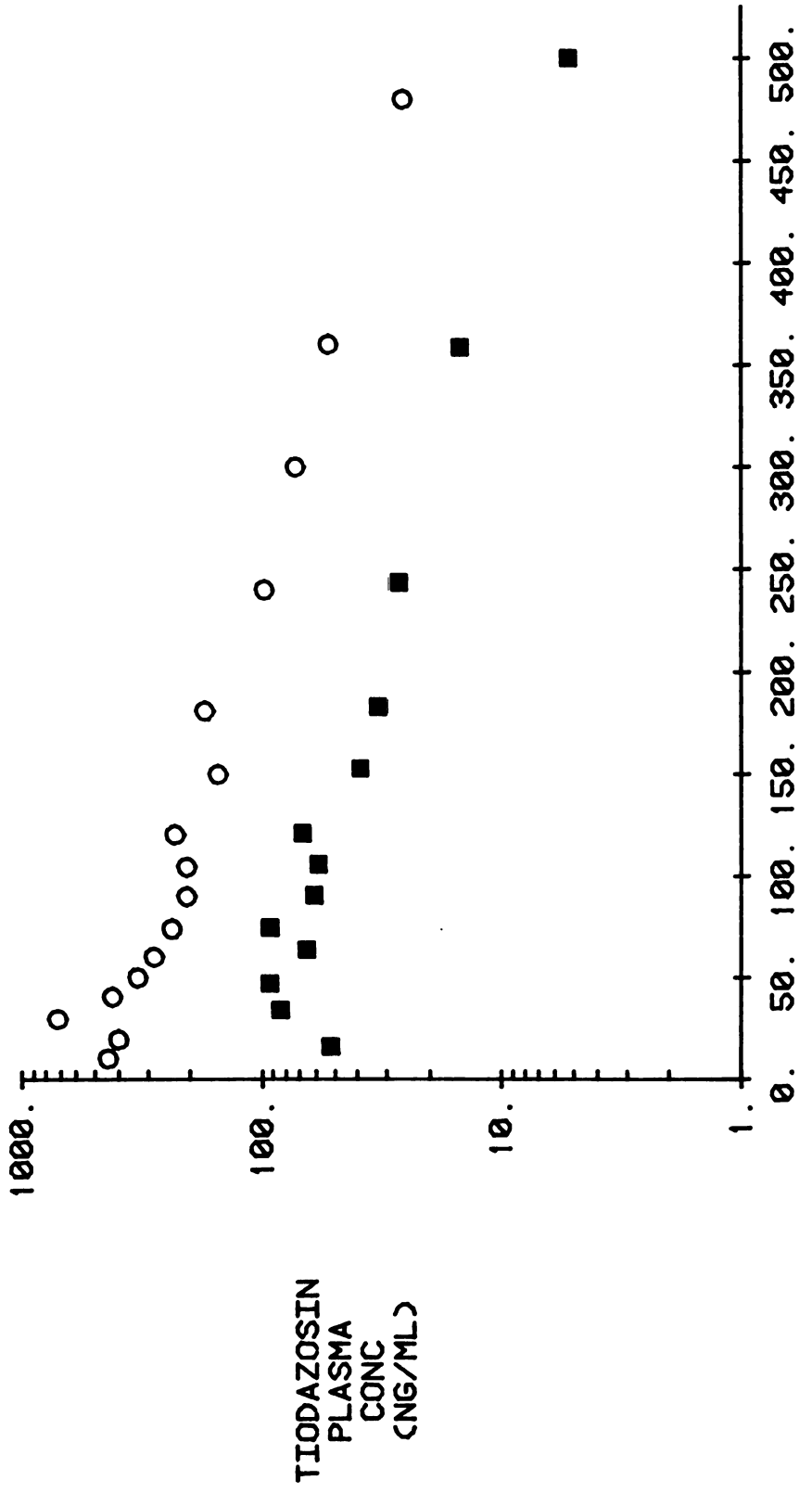

Figure A-1. Tiodazosin plasma concentration-time profile in one animal (28757) following a 30 minute intravenous infusion (○) and an oral solution (■) through a stomach tube. Samples were taken to 1440 minutes.

Table A-1. Tiodazosin pharmacokinetic parameters found in dogs following intravenous and oral dosing.

		Intravenous Dosing					Oral Dosing				
DOG #	WT (kg)	$Dose_{iv}$ (mg)	$t_{½z}$ (min)	$AUC^{\infty}_{0}{}_{B}$ (μg·hr/L)	V_{ss} (L)	CL_B (L/hr)	$Dose_{po}$ (mg)	$t_{½z}$ (min)	$AUC^{\infty}_{0}{}_{B}$ (μg·hr/L)	F_{pred}[a] (%)	F_{exptl}[b] (%)
28757	14.0	13.7	108	2202	17.6	6.22	12.4	134	477	76	26
29601	11.2	11.1	154	2163	14.7	5.13	11.2	95	280	75	13
28728	12.5	12.2	145	1358	35.6	8.98	13.4	83	513	61	32
29602	12.5	12.4	121	2006	19.1	6.19	12.4	160	720	73	34
28767	15.2	15.1	122	1941	23.6	7.81	15.1	142	479	72	24
MEAN	13.1	12.9	130	1934	22.1	6.87	12.9	123	494	71	26
± SD	1.5	1.5	19	340	8.2	1.76	1.5	32	156	6	8

[a] Equation IV-3

[b] Equation IV-1

mean predicted availability of tiodazosin in the five dogs was 71 ± 6% (range: 61 - 76%). A comparison of the mean data for tiodazosin to that previously determined for prazosin in beagle dogs, Table A-2, reveals that differences in the pharmacokinetics for these two members of the aminodimethoxyquinazoline class of vasodilators do exist. The mean half-life of prazosin following intravenous administration (192 ± 31 minutes) was significantly statistically different from the half-life following iv tiodazosin dosing (130 ± 19 minutes) ($p<0.01$). The half-lives of these two agents following oral administration were not statistically significantly different, but prazosin did show a trend to a longer interval (158 ± 32 min vs 123 ± 33 min). The most notable difference is the measured bioavailability. Tiodazosin demonstrated a mean F of 26% (range: 13 - 34%) which is roughly one-third that value for prazosin ($p<0.001$) at the same dose. All other values compared similarly. Statistical comparisons of the data from the four animals common to each study, paired t-test, were comparable with the results obtained from the unpaired analyses.

C. Discussion

Tiodazosin is a recently developed compound that is structurally similar to the vasodilator prazosin. Comparative studies involving these two compounds have demonstrated

significant differences between them in dogs. Lacking an intravenous dosage form for human trials, this work was performed in the animal model that has been traditionally used in studying these compounds so as to obtain an estimate of bioavailability, and the non-compartmentally determined disposition parameters.

The results in the beagle dog have shown that the terminal half-life for tiodazosin is significantly shorter than the prazosin terminal half-life. Since half-life is a derived parameter which varies as a function of clearance and volume of distribution:

$$t1/2 \cong (V \times \ln 2) / CL \qquad (A\text{-}1)$$

and because the two compounds demonstrated equivalent clearances, the shorter half-life of tiodazosin compared to prazosin can be accounted for by the variation in the volume parameter only (see Table A-2).

Based on the blood clearance of the compounds following intravenous administration and the estimated hepatic blood flow in dogs, predicted bioavailabilities for tiodazosin and prazosin (Equation IV-3) were essentially the same (73 and 71%, respectively).

The most unexpected finding from this experiment is the three-fold difference in the calculated bioavailability of tiodazosin compared to its predicted value, whereas the prazosin bioavailability at this high dose (1 mg/kg) is in good

Table A-2. Comparison of MEAN (± SD) pharmacokinetic parameters for prazosin and tiodazosin.

	Prazosin (n=4)	Tiodazosin (n=5)
WEIGHT (kg)	14.1 ± 2.0	13.1 ± 1.5
IV DOSE (mg)	14.3 ± 1.7	12.9 ± 1.5
CL_B (L/hr)	6.95 ± 0.74	6.75 ± 1.28
V_{ss} (L)	31.6 ± 2.7	22.1 ± 8.2
$t_{½_z}$ (IV)[a] (min)	192 ± 31	130 ± 19
ORAL DOSE (mg)	16.3 ± 2.5	12.9 ± 1.5
$t_{½_z}$ (PO) (min)	158 ± 32	123 ± 33
PLASMA:BLOOD RATIO	0.57 ± 0.04	0.61 ± 0.12
$F_{experimental}$[b] (%)	77 ± 11	26 ± 8
$F_{predicted}$[c] (%) (MEAN)	73	71

[a] $p<0.01$

[b] $p<0.001$

[c] Assuming an hepatic blood flow of 31 ml/min/kg (148)

agreement with its predicted value. These figures are even more notable when attention is given to the dosage forms used in the different studies. Prazosin was administered as either 3 or 4 commercially available capsules per animal. Tiodazosin was administered in solution via a stomach tube. If differences were to exist based on the dosage form, one would predict that the compound in solution would provide the higher availability, as it would not be subject to loss secondary to irregularities in disintegration and/or dissolution. Thus, with such a significant difference in the predicted and observed bioavailability for tiodazosin, the assumptions for the prediction of the extraction ratio for tiodazosin are incorrect. Preliminary data indicates that less than 2% of either compound is excreted unchanged in the urine. Therefore it appears as though tiodazosin is not completely absorbed from the dog gi tract and/or is degraded upon passage through dog gi membranes.

Possible mechanisms for the unpredictably low availability for tiodazosin include: 1) the precipitation of the compound upon contacting the acidic media in the stomach, with subsequent poor re-dissolution, and 2) a differential gastrointestinal metabolism with the variability between agents being determined by the difference in structures.

However, tiodazosin bioavailability may also be dose-dependent. The saturation of the pre-systemic metabolism may occur at a dose higher than that demonstrated for prazosin.

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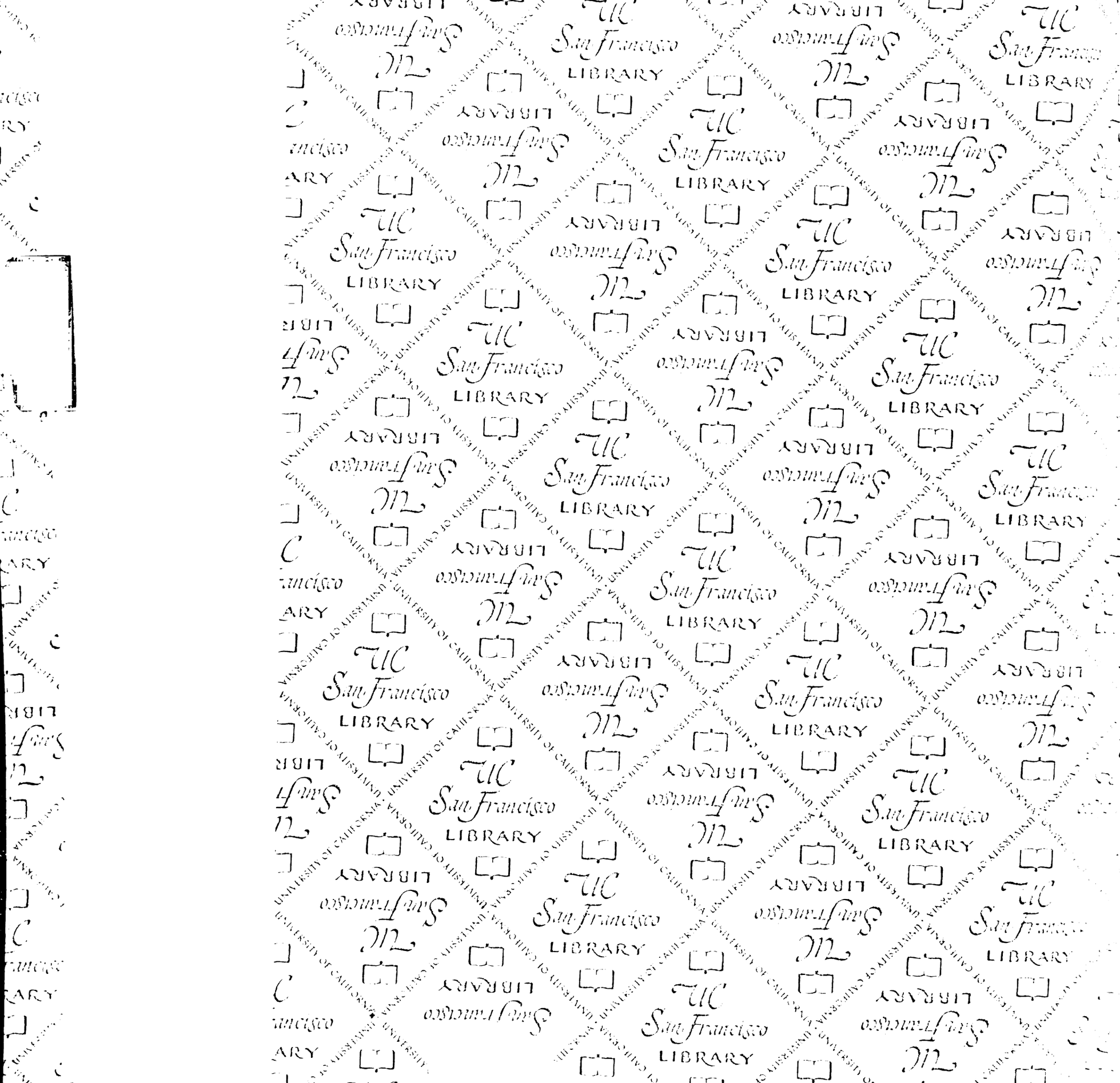
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