THE PHARMACOKINETICS AND PHARMACODYNAMICS OF CALCIUM ANTAGONIST DRUGS IN CARDIOVASCULAR DISEASE - INFLUENCE OF AGE, DISEASE STATE AND RENAL FUNCTION.

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF MEDICINE, UNIVERSITY OF GLASGOW

BY

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PREFACE

Over the past four years, spent in the Department of Materia Medica (Department of Medicine & Therapeutics) University of Glasgow, it has been a good opportunity to be involved in a series of clinical studies looking at the effects of calcium antagonist drugs in cardiovascular disorders. This afforded me an opportunity to use computer models such as statistical, pharmacodynamic and pharmacokinetic analyses to explain and present my results.

I was primarily responsible for conducting and analysing the studies described and the preparation of this thesis was entirely my own work.

Jawad H. Ahmed

November, 1989.

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I am also grateful to all members of the Department particularly Mr Daniel McSharry for drug analysis, Jim McCulloch for the determination of hormones and Dr. E. Farish for the analysis of lipids and lipoproteins.

I have enjoyed the friendship and the generosity of the staff at Stobhill hospital and the folk of the city of Glasgow.

I am indebted to the University of Basrah, Iraq, and the Ministry of Higher Education & Scientific Research for

their grant which enabled me to embark on this project.

Finally, I would like to express my sincere gratitude to my wife, Karima, and my children Haider, Naba and Ban for their inspiration.

SUMMARY

SUMMARY

This thesis has investigated several aspects of the clinical pharmacology of calcium antagonist drugs.

The first study, in patients with mild to moderate essential hypertension, investigated the pharmacokinetics of verapamil, the concentration-effect relationship of the antihypertensive response and some of the possible determinants of response using concentration-effect modelling. Thus the antihypertensive effect was characterised as "responsiveness" in mmHg/ng/ml, which is the magnitude of blood pressure reduction per unit change in plasma drug concentration, and is derived from the slope ("m") of the concentration-effect relationship. Responsiveness was closely correlated with the height of pretreatment blood pressure but not independently with plasma renin activity or age. The results of this study suggest that the reported increased responsiveness in elderly hypertensives, which was correlated with increasing age and decreasing plasma renin activity, may have failed to take account of the height of pretreatment blood pressure. Since there is an age-related increase in blood pressure there is no substantive evidence for an independent role for age or plasma renin activity in the antihypertensive responses to calcium antagonist drugs. This study confirmed also the accumulation of verapamil with multiple doses, with an increase in the area under the concentration time curve and a prolongation of the terminal elimination half life.

In addition to the reports that the elderly are more

responsive to the effect of calcium antagonists, there is some evidence that the pharmacokinetics of calcium antagonist drugs are altered in the elderly, with increases in plasma drug levels. Increased plasma levels themselves obviously might lead to an increase in therapeutic effect but also might increase the risk of toxicity. Potentially increased toxicity was a particular component of the study in patients with stable angina pectoris receiving single and chronic doses of verapamil (oral and intravenous). There were no significant effect of verapamil on left ventricular ejection fraction (EF%) or atrio-ventricular conduction (PR interval). Verapamil had no significant effects on ERPF, GFR or apparent liver blood flow. The results of this study indicate that there was no increased susceptibility to the negative inotropic or dromotropic effects of verapamil in middle aged and elderly patients.

The effect of age on the pharmacokinetics of verapamil was investigated for the pooled data on 74 subjects (age range 19-79) by the application of linear regression analysis across the age range. The results of this study suggest that increasing age is associated with small but significant alterations in the pharmacokinetics of verapamil. However, the variability in the pharmacokinetic parameters of verapamil also increased with age which makes it inappropriate to make global dosage recommendations for elderly patients by virtue of age alone.

Since calcium ions are involved in many biological processes, it is appreciated that calcium antagonist drugs

are likely to have more than one target tissue where they can exert a pharmacological effect. However, not all effects of calcium antagonists can be explained solely on their calcium channel blocking activity. For example, their potential role as adjuvant agents in the treatment of cancer with chemotherapeutic drugs. In vitro verapamil has proved a very effective agent but, unfortunately, the concentrations of racemic verapamil required to overcome drug resistance in vitro are relatively high and ranged between 2-6 μM (1000-3000 ng/ml). Such high concentrations are unlikely to be achieved in man without serious cardiovascular side effects particularly on atrioventricular conduction. Therefore, aiming to reach the required concentrations, we have undertaken a clinical safety and tolerability study using the less potent single isomer d-verapamil which has adjuvant activity similar to the racemic formulation but has less cardiotoxicity. The results of this single dose study showed that the required high concentrations of verapamil could be achieved with a side effect profile of prolongation of PR interval and reduction in blood pressure which was no worse than that after the racemic mixture. It has been speculated that sustained high drug levels might be achieved if d-verapamil is administered in multiple dose. Prior to conducting a steady state clinical study the concentrations likely to be achieved at steady state were predicted from the single dose data to provide us with a guide to the dosage regimen required. The 1000 mg dose was avoided because of its single dose toxicity but the predictions showed

that 500 mg d-verapamil three or four times daily might be appropriate to reach the required levels and maintain them for most of the period of dosage intervals.

Chapter 7 explores another aspect which would not readily have been expected from basic pharmacological principles. In renal impairment, many investigators have concentrated upon the effects of calcium antagonists on renal function and have largely ignored the elimination of these drugs on the basis that they are extensively metabolised in the liver. Although, nicardipine is extensively metabolised in the liver with less than 3% of the parent drug excreted unchanged in urine, in this study it was found that the plasma clearance was significantly reduced in patients with renal impairment but restored towards normal in patients undergoing regular haemodialysis. The reduction in plasma clearance was significantly correlated with renal function. The underlying mechanism is unknown. However, there were no changes in protein binding or volume of distribution; accumulation of renally-eliminated metabolites is also unlikely since the generation of metabolite is very limited after intravenous dosing. Restoration of the plasma clearance of nicardipine by haemodialysis has therefore been attributed to the removal, by haemodialysis, of some "inhibitory substances". This study emphasises the importance of fully investigating the pharmacokinetics of drugs in patients with renal failure even if the kidney is not the major site of elimination.

The final study (chapter 8) was undertaken to evaluate

the metabolic and hormonal responses to food and exercise after 4 weeks treatment with nicardipine. This "provocative" approach is seen as complementary to the conventional approach which depends only on baseline levels in investigating drug induced effects on serum lipids and hormones. The results of this study confirm that short-term treatment with nicardipine had no effect on the profile of lipids and hormones.

In conclusion, calcium antagonist drugs are likely to make an increasingly important contribution to the effective treatment of cardiovascular disorders. They have no adverse impact (perhaps even a beneficial effect) on end-organ blood flow, such as kidney or liver blood flow; they have a neutral effect on serum lipid and hormone profiles; they have no serious age-related toxicity; and, potentially, their use may be extended to the field of cancer research as adjuvant agents to overcome drug resistance.

CHAPTER ONE

INTRODUCTION

1.1 HISTORICAL REVIEW

Calcium antagonists do occur naturally. One such example is "tanshinone", which is derived from the root of a plant called "Salvia Miltiorrhiza Bunge", and which has been used in Chinese traditional medicine for centuries for the treatment of coronary disorders. In addition to plant sources, animal sources also have been identified such as in bee stings or from a tiapan snake venom. In traditional medicine, the therapeutic benefit of drugs is more important than a knowledge of the mechanism of action which, for calcium antagonist drugs, remained unexplored until about twenty years ago. While investigating the properties of two synthetic coronary vasodilator drugs, prenylamine and verapamil, Albert Fleckenstein and his colleagues observed that vasodilatation was accompanied by a negative inotropic effect which could then be reversed by the addition of calcium (Fleckenstein, 1971). From these experiments they concluded that these drugs inhibited the excitation-induced influx of calcium from outside to inside the cell.

Since the discovery of calcium antagonists came shortly after the introduction of beta blockers for the treatment of hypertension and angina, their comparably negative inotropic effect gave rise to the misconception that calcium antagonist drugs possessed beta blocking activity. Although, it then took only a very little time to establish that these drugs lacked beta adrenoceptor blocking activity their peripheral vasodilator action was largely ignored until the mid 1970's, when Lewis et al. (1978) first described a

sustained reduction in mean arterial pressure in a group of hypertensive patients receiving verapamil. However, by this time the potential use of calcium antagonists in the management of other cardiovascular disorders, including supraventricular tachycardia (Schamroth et al., 1972; Krikler and Spurrell, 1974) and angina pectoris (Livesley et al., 1973; Parodi et al., 1979), had attracted widespread attention.

1.2. THE ROLE OF CALCIUM IN EXCITATION-CONTRACTION COUPLING

Two contractile proteins are fundamental to the contractile process in both myocardium and vascular smooth muscle. The thin filament, actin, is a globular protein which polymerises to form a double helical structure and the second filament, myosin, is a hexamer with one pair of heavy chains and two pairs of light chains arranged in parallel to form a thick filament. At regular intervals, a portion of the myosin molecule projects from the thick filament and muscle contraction occurs when this globular portion of the myosin molecule attaches to, and detaches from, the actin filaments.

During relaxation, the concentration of calcium ions in the extracellular space is several order of magnitude higher than the concentration inside the myocardial or vascular smooth muscle cells.

During activation, the intra cellular calcium concentration increases markedly and calcium ions bind to a specific subunit of a regulatory protein (troponin in the

case of the myocardium; calmodulin in the case of vascular smooth muscle). This process initiates the interaction between the myosin bridge and the thin actin filaments so that the filaments are drawn toward the centre of the sarcomere resulting in the development of tension of the muscle and/or shortening (Figure 1.1.). The process of relaxation which follows the contraction results from reduction in the intracellular concentration of calcium leading to dissociation of calcium ions from troponin, or calmodulin, and subsequent breakage of the actin and myosin cross-link.

THE ACTION POTENTIAL-ROLE OF CALCIUM

Influx of calcium ions (Ca^{2+}) also plays an important role in the generation of the action potential of the heart. During phase 1 of the action potential, when depolarisation of the cardiac cell occurs, the membrane permeability to Na^{+} increases rapidly. The influx of Na^{+} through this so called "fast channel" in the cell membrane will result in a fast inward current. When the cell has been depolarised from approximately -90 to -40 mV a second inward current begins. This second current, which is due to the influx of Ca^{2+} is relatively slow compared to the fast Na^{+} current (Figure 1.2.). Although, the amount of Ca^{2+} ions which enter the myocardium during each depolarisation is small and may be not sufficient to initiate contraction, it probably triggers the release of Ca^{2+} from the intracellular sequestered stores (Braunwald et al., 1982).

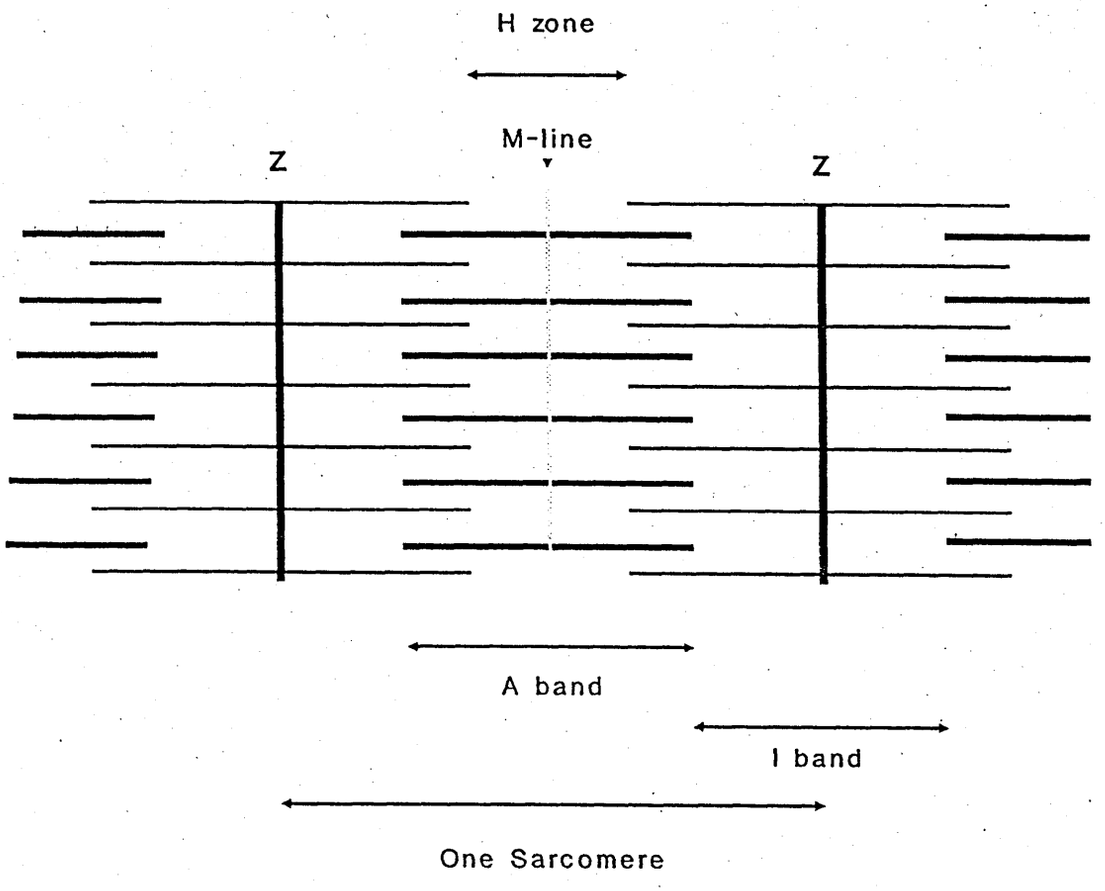


Figure 1.1. The relationship between the actin and myosin filaments in cardiac muscle. The A bands refer to the myosin filaments and the I bands to the actin filaments. The H zone refers to the myosin filaments when not overlapped with actin.

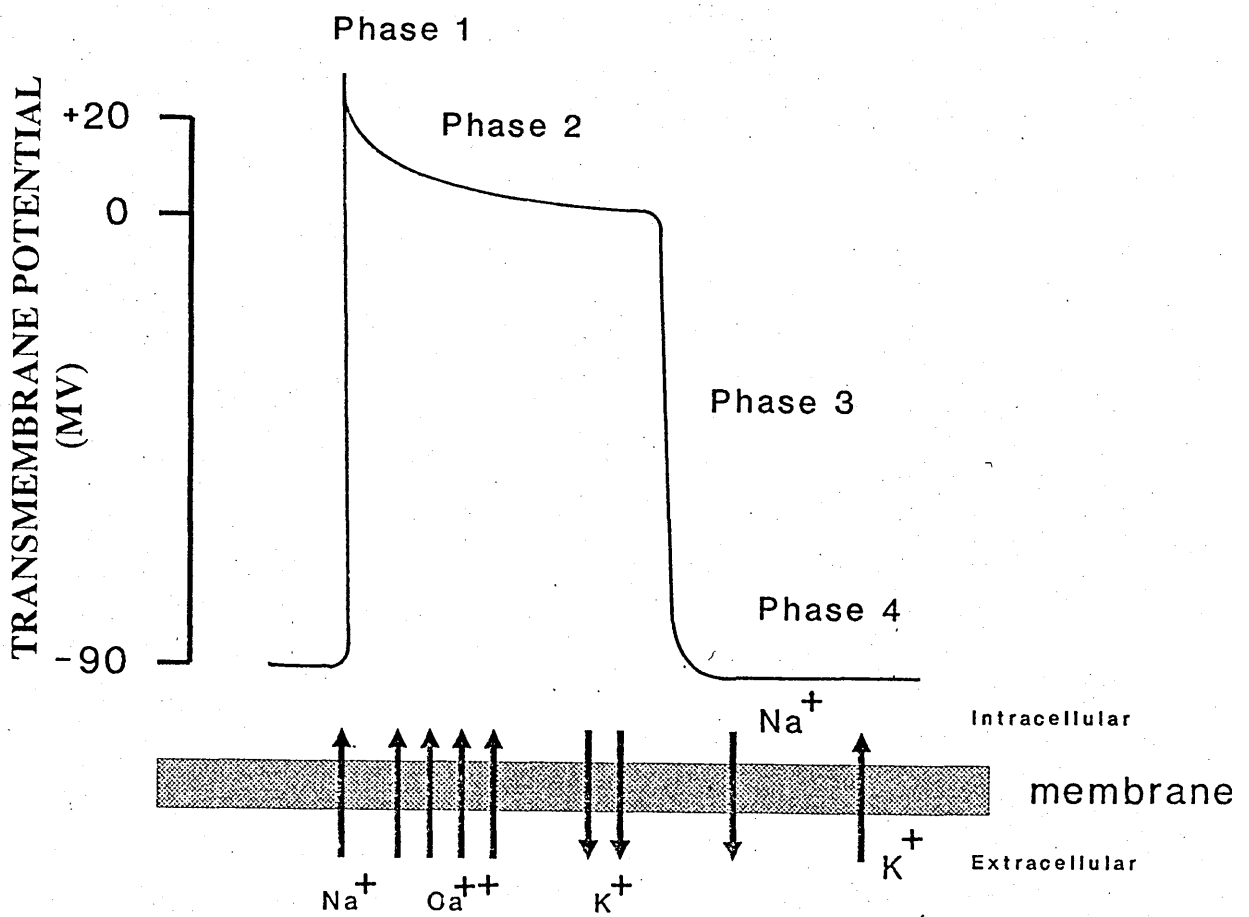


Figure 1.2. Schematic representation of the movement of ions during action potential of the heart; phase 0 represents the influx of Na^+ through the fast Na^+ channel and the plateau (phase 2) reflects the entry of Ca^{2+} ions through the Ca^{2+} channels; phase 3: repolarization; phase 4: diastole.

CONTRACTION OF MYOCARDIUM AND VASCULAR SMOOTH MUSCLE-ROLE OF CALCIUM

Although the contraction of myocardium and of vascular smooth muscle is similar in terms of the involvement of actin and myosin there are fundamental differences in the biochemical processes which regulate the process of contraction (Figure 1.3.). Contraction of vascular smooth muscle results from a series of reactions involving a small (15,000-Dalton) binding protein, calmodulin (Braunwald et al., 1982) to which Ca^{2+} ions bind when the intracellular Ca^{2+} concentration is approximately 10^{-6} M to form Ca^{2+}-calmodulin complex which activates the enzyme myosin kinase; this in turn phosphorylates a light chain of myosin leading to the interactions with actin resulting in smooth muscle contraction and arterial constriction. In the myocardium the calcium binds to a specific subunit of regulatory protein called troponin.

EXCITATION-SECRETION COUPLING-ROLE OF CALCIUM

Calcium is essential not only for muscle contraction but also for hormone release . This was first described by Douglas and co-workers (Douglas and Rubin, 1963; Douglas and Poisner, 1964) and confirmed by recent studies using calcium "activator drugs", such as Bay K8644, to increase the influx of calcium into the cell and produce an increase in the release of catecholamines (Montiel et al., 1984), or insulin (Panten et al., 1985). However, there is no good evidence

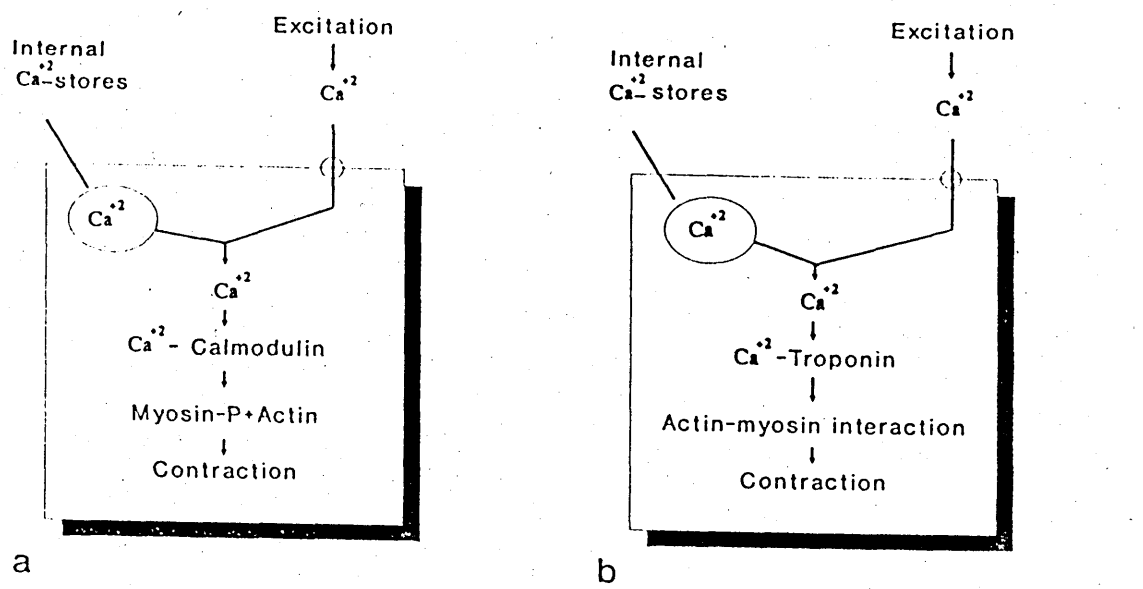


Figure 1.3. Schematic representation of the involvement of Ca^{2+} ions in the excitation-contraction coupling in (a) smooth muscle and (b) cardiac muscle.

that calcium antagonists in clinical doses have an inhibitory effect on the release of hormones (Rojdmark et al., 1979; Anderson et al., 1980).

Unlike the excitation-contraction coupling process, the detailed role of calcium in the biochemical processes of excitation-secretion coupling is not well defined.

CALCIUM CHANNELS

Until recently the molecular properties and classification of calcium channels were not defined but technical advances have facilitated the conduct of detailed studies to isolate the calcium channels and it is now possible to record the electrical activity associated with opening and closing of a single channel (Reuter et al., 1983).

The calcium channel is described as a macromolecular protein that traverses the lipid bilayer of the cell membrane and selectively permits the movement of Ca^{2+} ions from one side of the membrane to the other. Calcium channels have been classified into 3 major groups- L, T and N (Figure 1.4.). L, stands for long-lasting large-capacitance; T, stands for transient and N, for neuronal (Spedding et al., 1987). These three types of calcium channel differ from each other in terms of:

1. Voltage: The T-type is activated at relatively low potential (from -110 to -70 mV), L- and N-type are less sensitive to depolarisation and high potential is required for their activation (-110 to -10 mV) (Hagiwara et al.,

1983).

2. a) Sensitivity to organic and inorganic calcium antagonists, such as Co^{2+} and Mn^{2+}: the L-type is strongly blocked by inorganic calcium antagonists, such as cadmium, and by organic calcium antagonists, such as verapamil, diltiazem and nifedipine (Nowycky et al., 1985).

b) Responsiveness to Bay K8644: the L-type can be stimulated by a calcium agonist, such as Bay k8644 (Nowycky et al., 1985a,b; Hess et al., 1985b) and by a beta-adrenoceptor agonist such as isoprenaline (Hagiwara et al., 1988).

3. Acceptance of barium ions as a charge carrier instead of calcium: N- & L-types but not the T-type can use Ba^{2+} (Nowycky et al., 1985a).

4. Responses to toxins: for example the T-type is not sensitive to neurotoxin (TaiCatoxin) whereas the L_m-type (m for muscle) is blocked by this toxin (Brown et al., 1987).

As these channels are highly specific for calcium ions it can be assumed that they have a filter mechanism to define and select appropriate ions. In addition, these channels require a site which is sensitive to the propagation of electrical stimuli so that depolarisation will decrease the electronegativity of the cell with subsequent activation (opening) of the calcium channels and entry of calcium. The channel will then close when the interior of the cell returns to its pre-activation level of electronegativity.

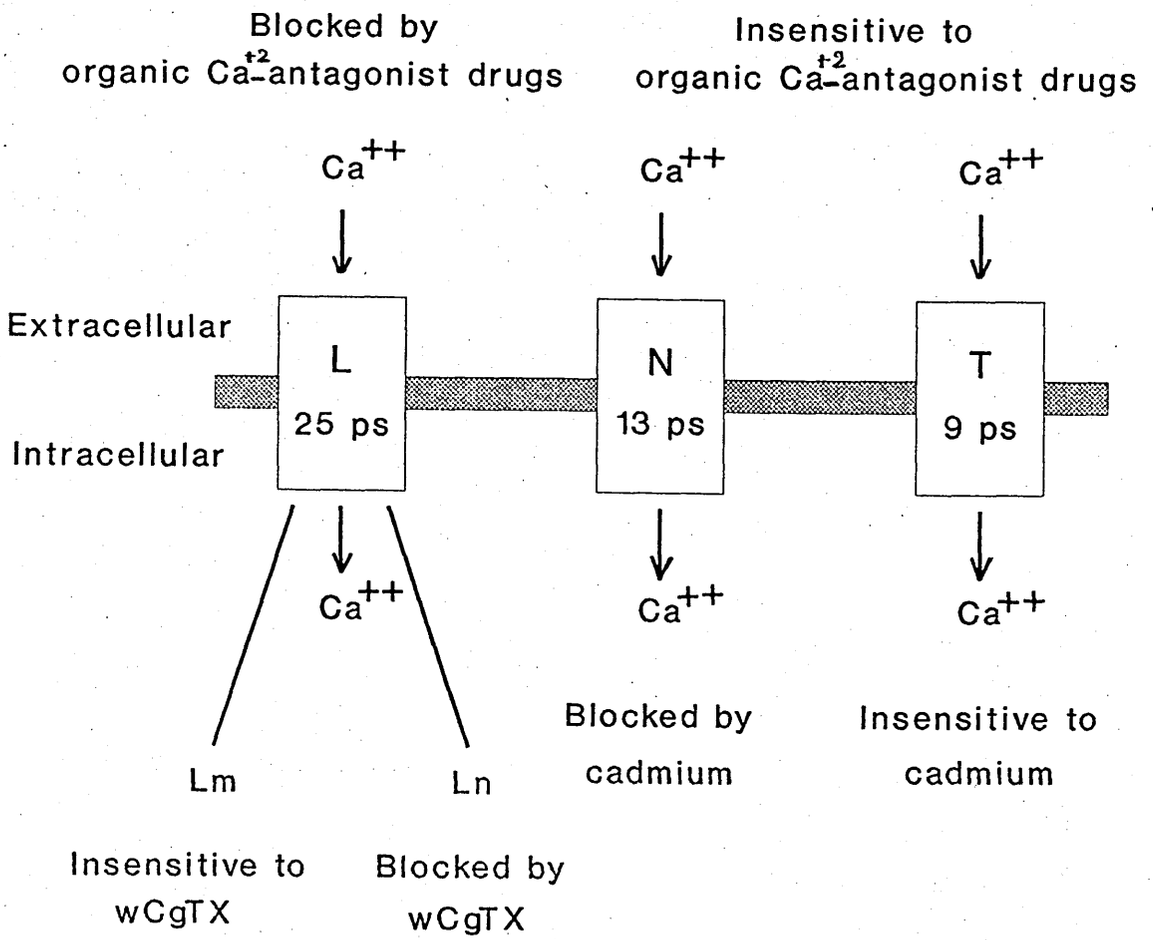


Figure 1.4. Types of calcium channels.
L, N, T : types of calcium channels.
Ps (Pico Siemens): channel conductance.
Lm : cardiac and smooth muscle L-channel.
Ln : neuronal L-channel.
wCgTX : omega-conotoxin.
(modified from Nayler, 1988)

Since the movement of calcium ions through these channels is controlled by electrical potential they have been termed "Voltage-dependent channel". In addition to their voltage dependency, however, calcium channels are also operated by membrane receptors, including beta1 and alpha2-adrenoceptors, and accordingly are named "Receptor-operated channels".

The distribution of these channels differs from one tissue to another. While some tissues have the three types of channel, other tissues including the myocardium, and particularly the cells of the pacemaker, contain only the "L" and "T" types (Hagiwara et al., 1988).

CELLULAR RECEPTORS FOR CALCIUM ANTAGONIST DRUGS

To produce its pharmacological effect a drug requires to bind to a receptor site. The specific binding sites for the different types of calcium antagonist drug have been studied by means of radio-labelling technique and saturable binding sites have been identified for the dihydropyridine, phenylalkylamines (verapamil), and the benzothiazepine (diltiazem) calcium antagonists (Triggle and Janis, 1984; Glossmann et al., 1987).

As with many other drugs, including the alpha and beta adrenergic blocking drugs, the binding sites of calcium antagonists may be affected by a number of different factors:

1. Age - Although there have been claims that elderly patients are more sensitive to the effect of calcium

antagonist drugs (Buhler et al., 1982) there is no conclusive evidence that the number of binding sites of calcium antagonists change with age. However, it has been shown recently that the density of binding sites (B_{max}) for dihydropyridine calcium antagonists in rat heart membrane preparation is increased (Dillon et al., 1989) with no change in affinity. The significance of this remains unclear.

2. Hypertension - A relatively small increase in the number of binding sites (using ^{3}H-nitrendipine) has been reported in hypertensive rats (Chatelain et al., 1984). No such evidence is available in man.

3. Ischemia - The number of binding sites has been reported to be reduced in ischemia (Dillon and Nayler, 1987).

4. Chronic therapy with calcium antagonists - It is well known that, for example, chronic administration of the beta adrenoceptor antagonist propranolol results in "up-regulation" (i.e. increase in the number of binding sites of beta adrenoceptors) (Glaubiger & Lefkowitz, 1977). The same effect has been investigated for calcium antagonists but no such change has been detected in the number of binding sites (Nishiyama et al., 1986). This could have clinical importance since withdrawal symptoms are rare after calcium antagonists.

TISSUE SELECTIVITY

One of the interesting features of calcium antagonist drugs is their relative selectivities for particular

tissues. For example, verapamil is effective not only on the peripheral vasculature but also on the myocardium and the conductive tissue of the heart, forming the basis of its use in controlling supraventricular tachyarrhythmias (Rowland et al., 1979). In addition, there is experimental evidence to suggest differential effects on different vascular beds. For example, in animal studies nitrendipine is highly selective for peripheral blood vessels, whereas nimodipine and nisoldipine are selective for cerebral and coronary blood vessels respectively (Kazda and Towart, 1981; Godfraind et al., 1987). It is not yet clear whether or not these differences in selectivity are due to differences in the distribution and type of calcium channels and their therapeutic relevance in man remains to be established. The relative tissue selectivities of the different calcium antagonists are summarised in table 1.1.

1.3. CLASSIFICATION OF THE CALCIUM ANTAGONISTS

Calcium antagonist drugs constitute a group of heterogeneous chemical compounds with the common property of an ability to inhibit the Ca^{2+} current. In addition to their chemical diversity, the differences in their tissue selectivity create difficulties for the construction of a useful clinical classification. Thus, the most commonly used classification, and the simplest, is related to the chemical classification. Accordingly calcium antagonists can be classified into 4 main groups (table 1.2.). The chemical structures of some calcium antagonist drugs are presented in figures 1.5., 1.6., 1.7.

TABLE 1.1.

TISSUE SELECTIVITY OF THE CALCIUM ANTAGONISTS

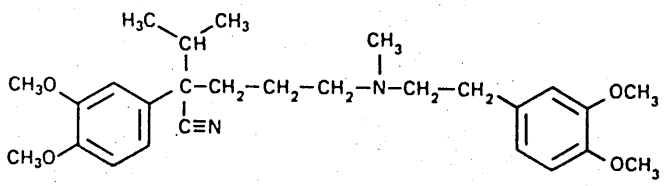
Drugs	**Heart (-ve inotropy)**	**blood vessels (Vasodilatation)**	**A-V node**
Verapamil	+	+	+
Gallopamil	+	+	+
Diltiazem	+	+	+
Nifedipine	+	++	-
Nitrendipine	+	+++	-
Nisoldipine	+	++++	-
Nimodipine	+	++++	-
Felodipine	+	++++	-
Amlodipine	+	++++	-
Cinnarzine	+	++++	-

+ refers to the presence of effect; and - to the absence of effect.

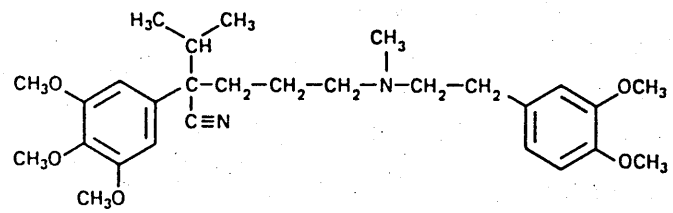
TABLE 1.2.

CLASSIFICATION OF CALCIUM ANTAGONIST DRUGS

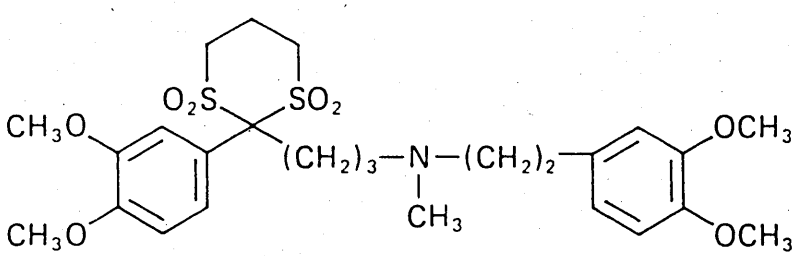
PHENYLALKYLAMINE	DIHYDROPYRIDINES	BENZOTHIAZEPINE	DIPHENYLALKYLAMINES
Amipamil	Amlodipine	Diltiazem	Cinnarizine
Desmethoxyverapamil	Azodipine	Fostedil	Flunarizine
Gallopamil	Dazodipine		Fendiline
Ronipamil	Felodipine		Prenylamine
Terodiline	Floridipine		
Tiapamil	Iodipine		
Verapamil	Isradipine		
	Nicardipine		
	Nifedipine		
	Niludipine		
	Nimodipine		
	Nisoldipine		
	Nitrendipine		
	Riodipine		
	Ryosidine		



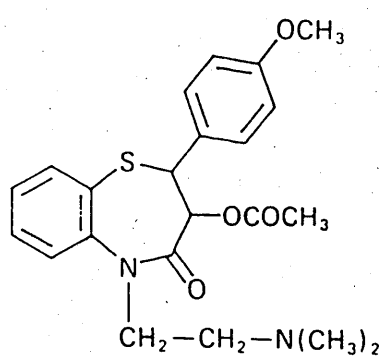
VERAPAMIL
(MW 454.54)



GALLOPAMIL
(MW 485.59)

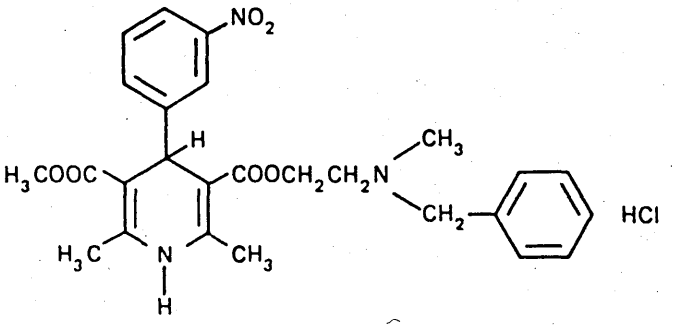


TIAPAMIL
(MW 592.10)

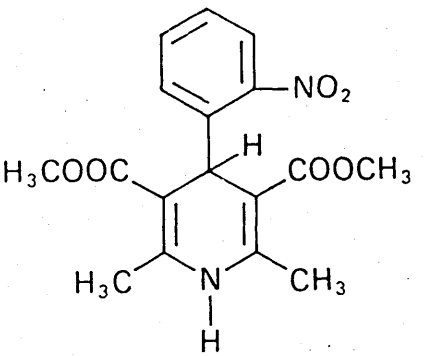


DILTIAZEM
(MW 414.52)

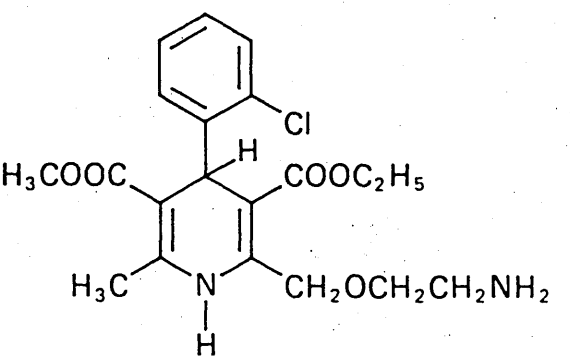
Figure 1.5. Chemical structure of calcium antagonists which exert effects on AV conduction.



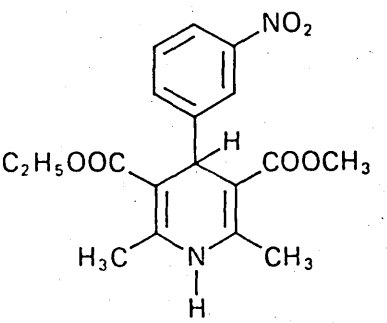
NICARDIPINE
(MW 388.42)



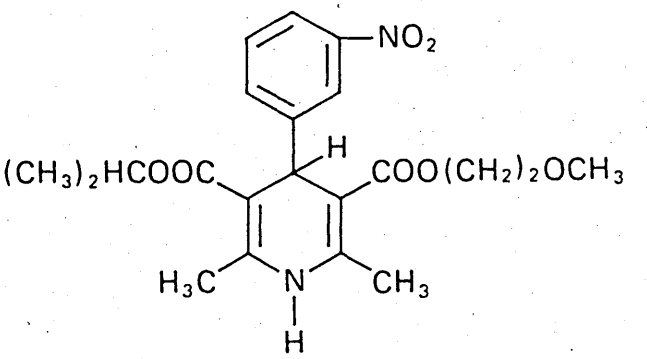
NIFEDIPINE
(MW 346.34)



AMLODIPINE
(MW 408.90)

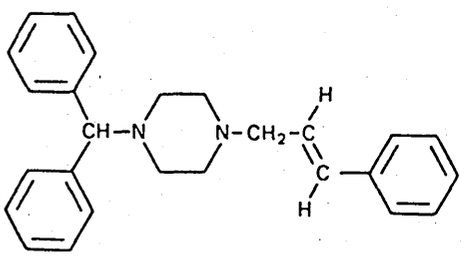


NITRENDIPINE
(MW 490.55)

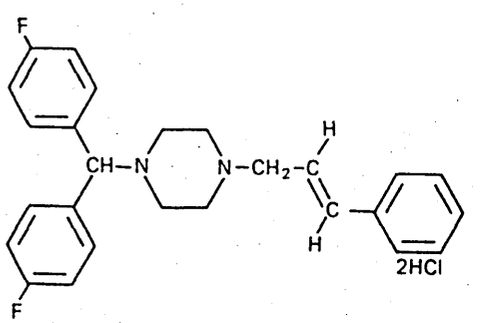


NIMODIPINE
(MW 418.45)

Figure 1.6. Chemical structure of dihydropyridine calcium antagonists



CINNARIZINE
(MW 360.50)



FLUNARIZINE
(MW 406.50)

Figure 1.7. Chemical structure of flunarizine and cinnarizine.

1.4. THE PHARMACOKINETICS OF CALCIUM ANTAGONISTS

1.4.1. VERAPAMIL

Verapamil [2,8-bis (3,4-dimethoxyphenyl)-6-methyl-2-isopropyle-6-azaoctanitrile] was the first calcium antagonist introduced to the field of medicine in the early 1960's by Fleckenstein. Because of the lack of sensitive and specific methods for the determination of plasma concentrations, it was not until the late 1970's that the pharmacokinetic characteristics of verapamil were fully described. A spectrophotofluorescence method was described by Koike et al. (1979) but this method cannot differentiate verapamil from its major metabolite and has restricted usefulness for single intravenous dosing. An HPLC method was developed by Cole et al (1981) and not only is it capable of differentiating between parent drug and metabolite but it also needs only a small sample size and short extraction procedures. This assay is sensitive to the level of 2 ng/ml.

PHARMACOKINETIC CHARACTERISTICS OF VERAPAMIL

Following oral administration, absorption from the gastrointestinal tract is almost complete (Schomerous et al., 1979) and the time to peak plasma concentration is about one hour after dosing. In normal subjects, verapamil is widely distributed throughout the body with an apparent volume of distribution in the range 2.5-4.8 l/kg after intravenous administration (Koike et al., 1979; Eichelbaum et al., 1981; McAllister et al., 1982).

Verapamil is extensively metabolised by the liver resulting in a low oral bioavailability of 10-20% (Eichelbaum et al., 1981; McAllister et al., 1982). Confirmatory evidence for the extensive first pass hepatic metabolism has been obtained from patients after mesocaval shunt operation in whom a bioavailability of 81% was observed (Eichelbaum et al., 1980 a).

Verapamil is metabolised in the liver by microsomal monooxygenase reactions catalysed by cytochrome P-450. Primary metabolic pathways of verapamil include N-dealkylation and O,N-demethylation (Eichelbaum et al., 1979) with the N-demethylated metabolite, norverapamil, as the principal identifiable metabolite following oral administration. In common with other drugs which undergo extensive first pass hepatic extraction there is a wide inter and intra individual variability in the extent of verapamil metabolism (Woodcock et al., 1980). In general, this variability is due to a variety of factors including nutrition, environment, concomitant drugs, exposure to chemicals, cigarette smoking, alcohol, disease state (Meyer et al., 1984) or genetic factors such as those described for nifedipine (Kleinbloesem et al., 1984).

One of the interesting features of verapamil pharmacokinetics is its accumulation with chronic dosing (Freedman et al., 1981; Shand et al., 1981). This is manifested as a reduction in clearance and a prolongation of the elimination half life (table 1.3.).

TABLE 1.3.

COMPARISON BETWEEN PHARMACOKINETICS FOLLOWING SINGLE AND MULTIPLE DOSES OF VERAPAMIL (MODIFIED FROM FREEDMAN et al., 1981)

	Single dose mean ± sd	**Multiple doses mean ± sd**	**P-value**
Verapamil $t_{1/2}$ (h)	5.7	9.6	<0.05
Verapamil t_{max} (h)	1.1 ± 0.3	1.0 ± 0.3	ns
Verapamil AUC (ng/ml*h)	378.0 ± 149.0	895.0 ± 309.0	<0.01
Verapamil Cl (ml/min)	4160.0 ± 1900	1750.0 ± 890	<0.01
Norverapamil $t_{1/2}$ (h)	8.0	12.0	<0.01
Norverapamil t_{max} (h)	1.4 ± 0.6	1.5 ± 0.5	ns
Norverapamil AUC (ng/ml*h)	590.0 ± 140.0	1084.0 ± 244.0	<0.01
Ratio Verapamil AUC/ Norverapamil AUC	0.6 ± 0.2	0.8 ± 0.2	<0.01

ns represents not significant; $t_{1/2}$ is the elimination half life; t_{max} is the time to reach peak plasma level; AUC is the area under the concentration time curve; Cl is the clearance.

PHARMACOKINETICS FOLLOWING SINGLE DOSE:

The reported mean terminal elimination half life ranges from 1.85 to 5.2 hours; the mean systemic clearance ranges from 875 to 1257 ml/min; the mean apparent clearance following oral administration ranges from 3746 to 6383 ml/min; and the mean apparent volume of distribution (Vd) ranges from 113 to 418 L. Norverapamil disposition has also been examined after single oral dose. Mean elimination half life ranges from 8 to 10.5 hours (Freedman et al., 1981; Kate et al., 1981; Schwartz et al., 1985).

PHARMACOKINETICS AFTER REPEATED DOSES:

Compared to single oral administration, there are significant differences in the disposition of verapamil during repeated doses. Typically these manifest as an increased bioavailability and decreased oral clearance, with a prolongation of the elimination half life (Freedman et al., 1981; Shand et al., 1981; Meredith et al., 1985; Schwartz et al., 1985). Apparent oral clearance (CL) during repeated doses was approximately half of that after a single oral dose. Eichelbaum (1984) has reported reduced apparent oral clearance but without significant prolongation of the elimination half life. To explain the differences in clearance and bioavailability after single and repeated administration a number of suggestions have been made:

1) Changes in hepatic blood flow

Because verapamil is highly extracted by the liver, its elimination is highly dependent upon changes in hepatic

blood flow.

It has been reported that liver blood flow (using indocyanine green clearance) increases after the first dose of verapamil and then declines towards placebo value with continued administration (Meredith et al., 1985). In association with these changes in apparent liver blood flow the clearance of verapamil may be initially relatively rapid with a subsequent decline during continued dosing. Changes in liver blood flow have also been reported with other calcium antagonist including nifedipine (Feely et al., 1984) and nisoldipine (Meredith et al., 1985; Van Harten et al., 1989). If the verapamil-induced change in hepatic blood flow is simply and directly related to verapamil plasma concentration, the maximum change in hepatic blood flow will be coincident with the peak plasma concentration but it will decline as plasma verapamil concentration declines. However, the extent of the increase in hepatic blood flow was attenuated with multiple doses in spite of the increases in C_{max} and AUC (Meredith et al., 1985). It seems likely that the contribution of verapamil induced changes in hepatic blood flow to the changes in verapamil pharmacokinetics is acute and transient.

2) Non linear (Saturation) pharmacokinetics (Wagner et al., 1984)

Wagner et al. (1984) studied the accumulation of verapamil at steady state by using published data (Freedman et al., 1981; Shand et al., 1981). Following oral administration, a relatively high concentration of verapamil

reaches the liver and saturates the enzyme system to produce a subsequent reduction in the plasma clearance of verapamil. If enzyme saturation does account for decrease in clearance then, as the plasma concentration falls again there should be a corresponding increase in clearance: this has not been reported to date. Similarly, there should be no accumulation of metabolite: however, although the principal metabolite norverapamil accumulates at steady state, this only provides evidence that the N-demethylation pathway does not undergo saturation and saturation of other pathways cannot be ruled out.

The concentration of verapamil in the portal circulation will be less after intravenous administration compared to that after oral administration and may not be so readily able to saturate the metabolic sites. However, this aspect will be dealt with in chapter 4.

3) Enzyme inhibition

There is increasing evidence to suggest that calcium antagonist drugs interfere with hepatic metabolic capacity. For example, the clearance of antipyrine was reduced by the concomitant administration of verapamil (Bauer et al., 1986; Rumiatsev et al., 1986; Batch et al., 1986) and, since antipyrine is entirely metabolised by the oxidative pathway, it has been suggested that verapamil inhibits hepatic enzyme activity. This has been substantiated by *in vitro* studies (Renton et al., 1985). Similarly, verapamil has been reported to inhibit the metabolism of carbamazepine (Macphee et al., 1986), digoxin (Johnson et al., 1987) and quinidine

(Edwards et al., 1987). Similar drug interactions have also been reported with other calcium antagonist drugs: nitrendipine and digoxin (Kirch et al., 1984), nisoldipine and digoxin (Kirch et al., 1986), diltiazem and antipyrine (Bauer et al., 1986), diltiazem and cyclosporin (Pochet et al., 1986; Carrum et al., 1986); nifedipine and digoxin (Kleinbloesem et al., 1985). Apart from the verapamil carbamazepine interaction (Macphee et al., 1986), most of the other reports investigated the interaction after 2 or 3 days of dosing with verapamil and consequently the onset of the interaction was not defined. In the verapamil-carbamazepine interaction, the onset of carbamazepine neurotoxicity and high plasma carbamazepine concentrations were detected 36-96 hours after the addition of verapamil and returned to normal 7 days after the withdrawal of verapamil. This evidence suggests that the metabolic sites need to be exposed to verapamil for a period of time before being inhibited and corresponding period of time is required for metabolism to be restored to normal. Since verapamil has the ability to inhibit the metabolism of other drugs which share the same metabolic pathway, it is highly possible that it may inhibit its own metabolism (autoenzyme inhibition) (Rumiatsev et al., 1986). Since the principal metabolite norverapamil accumulates at steady state it seems that the N-demethylation pathway does not undergo such autoinhibition.

1.4.2. NICARDIPINE

Nicardipine is a dihydropyridine derivative recently licensed in the U.K. for the treatment of angina pectoris and hypertension. The plasma concentrations of nicardipine have been measured using several quantitative techniques such as Gas-Liquid Chromatography with a sensitivity of 0.5-3 ng/ml (Clair et al., 1985), Gas Chromatography Mass Spectrometry with a sensitivity of 5 ng/ml (Higuchi and Kawamura, 1981) and HPLC which has a detection limit of 5 ng/ml and is capable of determining simultaneously nicardipine and its pyridine II metabolites (Wu et al., 1984).

Nicardipine is rapidly and completely absorbed after oral administration (Higuchi et al., 1977) and peak plasma concentrations are reached between 20 minutes and 2 hours after dosing.

In spite of the rapid and complete absorption of nicardipine from the gastrointestinal tract it has a low bioavailability (about 20%). Nicardipine is widely distributed throughout body tissues with an apparent volume of distribution of 63 ± 19 l in normal volunteers after intravenous administration (Campbell et al., 1985). In this study plasma concentrations declined in a bi-exponential manner with a terminal elimination half life of 59 ± 34 minutes and a plasma clearance of 1.2 l/min. This rate of plasma drug clearance corresponds approximately to the rate of liver blood flow.

There is also some evidence to suggest that the

metabolic processes for nicardipine may be saturable (Seki and Takenaka, 1977; Silke et al., 1984a). The maximum plasma concentrations (C_{max}) in 6 healthy volunteers given single oral doses of nicardipine 10, 20, 30 and 40 mg were 13, 32, 91 and 253 ng/ml respectively. However, no accumulation of nicardipine has been reported in 57 elderly hypertensive patient receiving 10-30 mg three times daily for 8 weeks (Higuchi and Shiobara, 1980a).

1.5. THE PHARMACODYNAMICS OF CALCIUM ANTAGONISTS

Calcium ions are involved in many biological processes, including myocardial and vascular smooth muscle contraction, and the smooth muscle contractions of the bronchial tree and of the gastrointestinal and urinary tracts. In addition to its effects on smooth muscle it also has a role in the secretion of hormones.

1.5.1. BLOOD PRESSURE AND HEART RATE

THE MECHANISM OF ACTION OF CALCIUM ANTAGONIST DRUGS IN HYPERTENSION

A characteristic feature of established hypertension is a raised peripheral vascular resistance (Lund-Johansen, 1980). Although the underlying mechanism is not clearly established, it has been proposed that, since cytosolic free calcium concentration is the final common trigger for phosphorylation of contractile proteins, there are close parallels between the contraction of myofibrils in smooth

muscle and the respective contractile proteins in other cells such as platelets. As it has been shown in patients with essential hypertension that platelet free calcium concentrations were elevated and directly correlated with the height of systolic and diastolic blood pressure (Erne et al., 1984; Asheley et al., 1986) the concept has arisen that defective "handling" of calcium may be involved in the genesis of hypertension (Aoki et al., 1976; Lederballe Pedersen, 1981; MacGregor et al., 1985; Buhler and Kiowski, 1987).

The antihypertensive action of calcium antagonist drugs mainly results from the vasodilatory effect which stems from the inhibition of entry of calcium ions into vascular smooth muscle cells, with subsequent reduction in the interaction between the contractile proteins. However, the "vasodilator" action is accompanied by several other factors which are relevant to cardiovascular regulation and may contribute to the net antihypertensive effect of calcium antagonists:

1. Increased cardiac output due to the reduction in after load which results from vasodilatation.

2. Reflex sympathetic activation (Millar et al., 1982; Littler et al., 1983) and activation of the renin-angiotensin system (Asplund et al., 1985; Baba et al., 1987).

3. Direct diuretic and natriuretic action (Young et al., 1985), although it seems unlikely that these have clinical significance since generally these effects are small and not sustained.

TREATMENT OF HYPERTENSION WITH CALCIUM ANTAGONIST

Calcium antagonist drugs have been shown to be very effective in the treatment of hypertension (Lewis et al., 1978; Midtbo and Hals, 1980; Gould et al., 1984) but their definitive position still remains to be established. Both verapamil and nifedipine reduce blood pressure significantly (Midtbo et al., 1982) but reflex sympathetic activation and increased heart rate are more common with nifedipine (Aoki et al., 1978; Lederballe Pedersen et al., 1980a) whereas the fall in blood pressure after verapamil is not usually accompanied by significant changes in heart rate or plasma renin activity (Leonetti et al., 1980; Corea et al., 1981).

Rapid falls in blood pressure have been reported after oral or sublingual doses of nifedipine (Guazzi et al., 1977; Jaker et al., 1989) providing a basis for its use in emergencies such as in the treatment of hypertensive crises.

Numerous reports have confirmed the antihypertensive efficacy of chronic monotherapy with calcium antagonist drugs (Midtbo & Hals, 1980; Anavekar et al., 1982) the magnitude of which is comparable to that of beta-blocking drugs (Doyle et al., 1983).

Calcium antagonist drugs can also be combined with other antihypertensive drugs. Although the combination of beta blockers with verapamil is not widely used the same combination with nifedipine offers potential advantages not only in efficacy but also in reducing side effects. Nevertheless, although nifedipine *in vivo* has less cardiodepressant activity than verapamil, some caution

concerning its potential cardiodepressant activity may be necessary in combination with beta-blockers particularly in those patients with impaired cardiac function (Singh et al., 1982).

Several other studies have reported clinically useful additive antihypertensive effects from the combinations with a thiazide diuretic (Labriola et al., 1978; Wicker et al., 1983) and with an alpha1-antagonist (Jee and Opie, 1983; Pasanisi et al., 1984). Similarly, the combination of calcium antagonist drugs and angiotensin converting enzyme inhibitors has been shown to be effective, initially in treatment of severe and resistant hypertension (Guazzi et al., 1984; White et al., 1986) but more recently in a wider range of patients with mild to moderate hypertension (Donnelly et al., 1986; Lees et al., 1987).

TREATMENT OF HYPERTENSION IN THE ELDERLY

The need for effective, safe and well tolerated antihypertensive treatment for elderly patients with hypertension has become more apparent since the results of the European Working Party on Hypertension in the Elderly (EWPHE) trial showed the benefit of blood pressure reduction in this population (Amery et al., 1985).

The principal aim of the treatment of hypertension is to reduce the morbidity and mortality of cardiovascular disease. Since there is a correlation between increasing age and increasing blood pressure (Editorial (Lancet), 1981; Sower, 1987) the therapeutic approach to hypertension in the

elderly has naturally generated considerable interest.

Until recently, the benefit of antihypertensive treatment of this age group was uncertain. The selection of the most appropriate antihypertensive agent for elderly patients presented some problems since it was generally thought that the therapeutic window of most drugs was relatively narrow in this age group and that serious side effects might compromise the benefits of treatment. In addition, the age-related decreases in renal and hepatic function might result in higher drug concentrations with a potential further risk of increased toxicity. A further complicating factor might also be the presence of a concomitant disease state, such as congestive heart failure, obstructive airways disease, peripheral vascular disease, diabetes or renal failure.

Since calcium antagonist drugs are generally free of serious adverse or toxic effects, such as depression of central nervous system responses, they appear to be a useful possible treatment for the elderly. Furthermore, some early studies with calcium antagonist drugs suggested that they were particularly effective antihypertensive agents in the elderly, in patients with low plasma renin activity and in black patients (Buhler, 1988). However, more recent studies have shown that these drugs are equally effective in both young and elderly hypertensives (Ram, 1987; Montamat and Abernethy, 1989) and the apparently greater antihypertensive response in the older age group may well be related to their higher pretreatment blood pressures (Lederballe Pedersen et

al., 1980a; Meredith et al., 1987). However, there may be age-related qualitative differences in the antihypertensive response such as are related to diminished counter-regulatory responses (Gribbin et al., 1971).

1.5.2. A-V CONDUCTION

The conductive tissue in the heart is preferentially sensitive to the effect of verapamil and diltiazem resulting in prolongation of the P-R interval (Smith et al., 1983). It is not specifically clear, however, why the dihydropyridine derivatives lack this effect. The effect on the P-R interval is also influenced by several other factors:

1. Route of administration- Studies with verapamil have shown that the effect on P-R interval varies with the route of administration whereby the concentration needed to produce a 10% prolongation of P-R interval was 2-3 times higher after oral than after intravenous administration. A possible explanation for this difference is that the most pharmacologically active isomer, L-verapamil, is preferentially metabolised by the liver after oral administration (Eichelbaum et al., 1980 ; Johnson et al., 1981; Reiter et al., 1982) whereas, after intravenous administration, the two isomers D- and L-verapamil are metabolised equally. The overall effect, therefore, is that more L-verapamil is available after intravenous administration.

2. Age- Since it is possible that there might be increased drug levels in the elderly and since there is

evidence to show that prolongation of P-R interval is correlated with verapamil plasma concentrations (Eichelbaum et al., 1980) it might have been expected that more pronounced prolongations of P-R interval would occur in the elderly. However, Abernethy et al. (1986) have shown that the P-R prolongation was less pronounced in elderly hypertensive patients compared to young hypertensives treated with verapamil. This implies that the sensitivity of the conductive tissue is decreased in the elderly. In a comparative study in a group of young and elderly subjects, diltiazem has been shown to be less effective on PR interval in the elderly although there was no age-related differences in the pharmacokinetics between the two groups (Montamat and Abernethy, 1989).

3. Other factors- Heart rate, sympathetic, parasympathetic and intrinsic baroreflex activity can influence the atrioventricular conduction responses to calcium antagonists. For example, the P-R interval shortens in relation to an increase in heart rate or sympathetic activity.

1.5.3. INOTROPIC EFFECT

All calcium antagonist drugs have negative inotropic effects on isolated cardiac muscle due to their inhibition of the transmembrane influx of calcium necessary for activation of the cross-bridges between actin and myosin. In humans, this effect depends on the route of administration of the calcium antagonists. For example, nifedipine has the

most potent negative inotropic effect when administered directly into the coronary arteries; diltiazem and verapamil are approximately equipotent when they are given by the same route (Nayler et al., 1983). However, if these drugs are given by the intravenous, oral or sublingual route, verapamil shows the most potent negative inotropic effect; and nifedipine is the least depressant; diltiazem is intermediate in action. Thus, in intact man, the overall effect on cardiac performance reflects the direct negative inotropic effects, vasodilatation with reduction in afterload as a result of improvement in myocardial perfusion and the resultant baroreceptor-mediated sympathetic stimulation of the myocardium. Nevertheless it is possible that the therapeutic benefits of these drugs, in the treatment of angina and hypertension, might be compromised in those patients who additionally have preexisting impairment of cardiac function.

The mechanisms by which calcium antagonists produce their different inotropic effects have been widely studied. In a study in guinea pigs, for example, the positive inotropic response to A 23187 (an agent which is known to transfer calcium into the cell without involving the calcium channels) was not influenced by nifedipine and was only slightly antagonised by verapamil and diltiazem (Boddeke et al., 1988). In contrast the positive inotropic effect of BAY K8644 (a calcium promoter through the dihydropyridine channel) was blocked by nifedipine, verapamil and diltiazem. These findings suggest that the negative

inotropic effect of calcium antagonists is not simply due to blockade of calcium entry but also involves other mechanisms at the cellular level, perhaps inhibition of calmodulin or inhibition of the release of sequestered calcium.

1.5.4. LIVER BLOOD FLOW

The liver is the central metabolic organ in the body and therefore liver blood flow and the integrity of hepatic enzyme systems are the major determinants of hepatic metabolic function. In recent years clinicians and pharmacologists have paid close attention to the problem and limitation of the techniques for assessing liver blood flow as it has been found that drug metabolism can be influenced by changes in liver blood flow.

Different methods have been described for the measurement of liver blood flow (Ohnhaus et al., 1979). Some of these methods are highly invasive and involve catheterisation of the hepatic vein (Woodcock et al., 1981) and thus are not suitable for research purposes. The indirect methods which depend on the clearance of a highly extracted marker such as indocyanine green, have the advantages of being non-invasive. Indocyanine green (ICG) is a sterile, water soluble, tricarbocyanine which is widely used and preferred because it does not undergo biotransformation in the liver and there is no evidence of enterohepatic recirculation. In addition reliable methods have been developed for its measurement using spectrophotometry or HPLC (Rappaport et al., 1982).

Studies with calcium antagonists have shown that verapamil, nisoldipine and nifedipine significantly increase liver blood flow (Feely et al., 1984; Meredith et al., 1985a,b). However, this increase in liver blood flow is particularly pronounced after acute dosing and declines towards baseline values with continued administration (Meredith et al., 1985a,b). The underlying mechanism responsible for this pattern of change is not clear but may reflect either increased cardiac output, consequent upon afterload reduction, or direct portal and splanchnic vasodilatation or both.

1.5.5. RENAL FUNCTION

Maintaining (ideally improving renal perfusion) is an important aim in the treatment of hypertension.

Reduction of blood pressure and cardiac contractility by calcium antagonists potentially might compromise renal function. However, because calcium antagonists are potent vasodilators renal blood flow is preserved, despite the reduction in blood pressure. This is a potential therapeutic advantage of calcium antagonists in patients with renal impairment since there is some evidence that betablockers (particularly propranolol) reduce renal blood flow and glomerular filtration rate (Warren et al., 1974; Webber and Drayer, 1980; Bauer et al., 1983). Thiazide diuretics are less effective when there is a moderate to severe renal impairment and angiotensin converting enzyme inhibitor may have adverse effects on renal function especially where

there is renovascular disease and the maintenance of adequate renal plasma flow is dependent on the renin angiotensin system (Hricik, et al., 1983; Curtis, et al., 1983). Not only do calcium antagonist drugs avoid reducing ERPF but there is evidence that renal plasma flow and glomerular filtration rate may in fact be increased, at least in the short term, in normotensive and hypertensive patients after the administration of nisoldipine, verapamil, nifedipine and nicardipine (Yokoyama et al., 1983; Van Shaik et al., 1984; Meredith et al., 1985a,b; Baba et al., 1986; Chaignon et al., 1986).

1.6. EFFECT OF AGE ON THE PHARMACOLOGY OF CALCIUM ANTAGONISTS

The increasing proportion of middle-aged and elderly patients in the general population has stimulated a specific interest in the factors which influence the responses of the elderly to various drugs, including the calcium antagonists. There are two basic factors which can modify the response to a drug: firstly, as already discussed (1.4), age-related changes in drug clearance, disposition and plasma concentration. Secondly, alteration to the response mediated via an "effector" site, either directly with respect, for example, to the number and affinity of the responsible receptors, or indirectly in terms of the extent of the compensatory cardiovascular responses.

Age related structural and physiological changes have been recognised which are associated with altered hepatic

drug metabolism in the elderly. Such changes include reduction in liver size and liver blood flow, in the synthesis of binding protein and in the capacity of the metabolising enzymes. Other factors such as nutrition, disease state, concomitant drug administration, genetics, smoking habit and environmental factors can additionally affect the metabolism of drugs in the elderly.

While there is a good evidence in man that liver blood flow declines with age (Bender et al., 1965; Wynne et al., 1988), there is only indirect evidence from pharmacokinetic studies to suggest a reduction of hepatic enzyme capacity. However, in animal studies an age-related reduction in hepatic metabolic capacity has been demonstrated (Kato and Takanaka, 1968). These age-related changes in liver function can affect drugs such as the calcium antagonists since the liver is the major site for their metabolism.

1.7. THE METABOLIC AND HORMONAL EFFECTS OF CALCIUM ANTAGONISTS

Several epidemiological studies have established that the prevalence and incidence of clinical ischemic heart disease in western countries are correlated positively with plasma total cholesterol and low density lipoprotein cholesterol (LDL) and negatively with the plasma high density lipoprotein cholesterol (HDL) (Miller et al., 1977; Hulley et al., 1980; Castelli et al., 1986). These findings have produced an increased awareness of the need to investigate the metabolic effects of antihypertensive drug,

on plasma lipids, especially for those drugs which have recently been licensed. The effects of different antihypertensive drugs on plasma lipid are summarised in table 1.4.

Beta blockers, including propranolol, and thiazide diuretics have been used for many years for the treatment of hypertension and have been proved effective in controlling high blood pressure. However, several large clinical trial have shown that treatment regimens based upon propranolol or thiazide diuretics have made no significant difference to the overall rate of coronary events, although the total cardiovascular events (which include stroke and coronary heart disease) are significantly reduced (MRC, 1985). This relative lack of therapeutic success raised the possibility that drug-induced adverse metabolic changes, particularly on serum lipids and lipoproteins, might be compromising the benefits of blood pressure reduction.

As described earlier in this chapter, since calcium ions have an established role in the processes which are responsible for the secretion of hormones, it is important to investigate the effect of calcium antagonist drugs on hormonal secretion and metabolic responses. A number of published studies have investigated the effect of calcium antagonist drugs on insulin secretion. No significant interference was found with verapamil (Rojdmark, 1979; Anderson and Rojdmark, 1980) or with nifedipine (Joffe et al., 1983) or nicardipine (Dow et al., 1985). Similarly, there was no significant effect on the secretion of

TABLE 1.4.

THE EFFECT OF ANTIHYPERTENSIVE TREATMENT ON PLASMA LIPIDS AND LIPOPROTEINS (MODIFIED FROM WEINBERGER, 1985)

Drug	total cholesterol	HDL	Triglyceride
Diuretics			
Chlorthalidone	↑	ns	↑
Hydrochlorthiazide	↑	ns	↑
Beta blockers			
Propranolol	↔↑	↓	↑
Atenolol	ns	↓	↑
Pindolol	ns	ns	ns
Calcium antagonists			
Nicardipine	ns		ns
Nifedipine	ns		ns
Verapamil	ns	ns	ns
Others			
Methyldopa	ns	↔↓	ns
Prazosin	↓	↔↑	↑↓
Captopril	ns	ns	ns

Row represent direction of effect; ns: not significant.

leutinizing hormone (LH), follicular stimulating hormone (FSH) or thyroid stimulating hormone (TSH) (Struthers et al., 1983; Isles et al., 1985).

1.8. THE SIDE EFFECTS OF CALCIUM ANTAGONISTS

Generally, calcium antagonist drugs are well tolerated. The most common side effects, which are an extension of the vasodilatory effects, are usually transient although occasionally they result in withdrawal of the drug. Thus, ankle oedema and headache are more common with nifedipine whereas verapamil is commonly associated with constipation and atrio-ventricular block (table 1.5.).

1.9. SCOPE OF THE THESIS

This thesis aimed to clarify some aspects of the clinical pharmacology of calcium antagonist drugs.

Detailed studies have been undertaken with verapamil and nicardipine. These studies have addressed several themes. For example the antihypertensive responses to verapamil and some of the possible determinants of the responses such as age, plasma renin activity, pretreatment blood pressure have been investigated in hypertensive patients using concentration effect modelling. The safety and the effect of verapamil on left ventricular function in middle age and elderly patients with stable angina has been investigated.

Using pooled data from 74 subjects receiving verapamil, age related changes in pharmacokinetics of verapamil have

TABLE 1.5.

THE REPORTED SIDE EFFECTS AFTER TREATMENT WITH CALCIUM ANTAGONISTS

Calcium antagonist	Side effect
Verapamil	Constipation (30%) Facial flushing (8%) Dizziness (6%) Headache (8%) AV block (1%) Worsening of heart failure
Nifedipine	Ankle oedema (15%) Facial flushing (12%) Headache (6%) Nausea (3%) Tachycardia (15%) Dizziness (3%)
Diltiazem	Headache (2%) Facial flushing (1%) Nausea (3%) Ankle oedema (2%) Constipation (22%)

The figures in parentheses are included to give an idea of the frequency with which these side effects are encountered (modified from Nayler, 1988).

been investigated.

The pharmacokinetics and pharmacodynamic of the single-isomer d-verapamil have been studied in healthy volunteers aiming to reach high plasma levels suitable for adjuvant activity to enhance the activity of anticancer drugs.

On nicardipine, two studies have been completed. One to evaluate its effect on serum lipids and hormones and the other on the effect of renal impairment and haemodialysis on the pharmacokinetics of nicardipine.

CHAPTER TWO

METHODS

2.1. GENERAL PROCEDURES

All protocols for the studies described in this thesis received prior approval by the Research and Ethical Committee of the Northern District of Greater Glasgow Health Board. All subjects gave written informed consent. For those studies which used radionuclides for the assessment of cardiac function or renal function subjects were particularly informed about their use. An ARSAC (Administrative of Radioactive Substances Advisory Committee) certificate is licensed to the supervisor, Dr. Elliott.

All subjects had routine clinical, biochemical, haematological and electrocardiographic examinations before starting each study and at the end of each study. Subjects reported to the clinical pharmacology research unit (C.P.R.U.) at approximately 08.30 in the morning having abstained from food, alcohol, nicotine and caffeine from 2200 on the night before the study day. An intravenous cannula was introduced into an antecubital vein for blood sampling. A fresh solution of heparin in normal saline was prepared for each subject and used to keep the cannula patent after blood collection. Thereafter, the subjects rested for at least 20 minutes before any recordings were taken.

2.2. ASSAY FOR DRUGS

2.2.1. DETERMINATION OF VERAPAMIL AND NORVERAPAMIL

Verapamil and norverapamil plasma concentrations were

determined by an HPLC method (Cole et al., 1981). This method has the advantages of being sensitive for detection of low plasma levels, employs simple extraction procedures and permits the simultaneous determination of verapamil and its major metabolite, norverapamil.

The mobile phase was a solution of potassium bromide (3 mM) and perchloric acid (0.37 mM) and was helium-degassed before use. A constant flow rate of 2.0 ml/min was maintained by a pressure of approximately 60 bar.

Sample preparation

Plasma or serum (100 µl) was pipetted into a small (Dreyer) test-tube (Poulton, Selfe and Lee, Wickford, Great Britain). Internal standard solution (50 µl), sodium hydroxide solution (50 µl) and methyl tert.-butyl ether (200 µl) were added using Hamilton repeating mechanism fitted with Hamilton gas-tight Luer-fitting glass syringes and stainless steel needles. The contents of the tube were vortex-mixed for 30 seconds and centrifuged at 9950 g for 2 minutes in an Eppadrof centrifuge. Subsequently, a portion (approximately 110 µl) of the extract was taken and used to fill the sample loop of the injection valve.

Samples were analysed in duplicate and the mean result was taken.

The intra-assay and the inter-assay coefficients of variation were less than 5% for both verapamil and norverapamil. The limit of detection of the assay was 2 ng/ml using a 100 µl sample.

2.2.2. DETERMINATION OF NICARDIPINE

The plasma concentrations of nicardipine were determined by an HPLC method developed by Wu et al. (1984). This method permits the simultaneous measurement of nicardipine and its pyridine metabolite. These assays were carried out in Syntex Research Laboratories, Riccarton Scotland.

2.3. PHARMACOKINETIC ANALYSIS

The pharmacokinetics of verapamil and its principal metabolite norverapamil were analysed both by model-dependent and model-independent means. The area under the concentration time curve to infinity (AUC) was calculated by application of the trapezoidal rule over the period of plasma sampling, plus the residual area. The area of the trapezoid is $1/2(C1 + C2) * \Delta t$. If Δt is too large (greater than 4 hours) the area of the trapezoid can be accurately measured by logarithmic trapezoidal rule so that:

$$\text{The area of the trapezoid} = \frac{(C1 - C2) * \Delta t}{\ln C1 - \ln C2}$$

Where C1 and C2 are two consecutive plasma concentrations, Δt is the time between these points and (ln) is the natural logarithm. From this, apparent oral clearance was determined by dividing dose by the $AUC_{0-\infty}$ for acute dosing and dose by the AUC_{0-T} for chronic dosing, where T is the dosage interval. Terminal elimination half-life was estimated from the log linear regression of the final concentration data

points. The verapamil and norverapamil concentrations were then analysed by model-dependent methods by fitting to a hierarchy of different models, the most appropriate model being selected by application of the General Linear Test (Boxenbaum et al., 1974). In all individuals the most appropriate model was an integrated model in which parent drug concentrations were fitted by a two-compartment model whilst the metabolite norverapamil was simultaneously fitted by a third compartment arising from the drug central compartment (figure 2.1.). The concentrations after the intravenous administration of verapamil were appropriately fitted by a two compartment model with a first order input to the central compartment (figure 2.2.). The concentrations after intravenous infusion were best fitted to a two compartment model of the form described by Gibaldi & Perrier (1975):

$$C = \frac{R_0 (K_{21} - \alpha)(e^{-\alpha T} - 1)}{V_c \alpha (\alpha - \beta)} e^{-\alpha t} + \frac{R_0 (\beta - K_{21})(e^{-\beta T} - 1)}{V_c (\alpha - \beta)} e^{-\beta t}$$

Where,

C = Plasma concentration (ng/ml)

R_0 = Infusion rate (μg/h)

K_{21} = Apparent first order mass rate constant associated with movement of the drug from the peripheral to the central compartment (h^{-1}).

V_C = Volume of distribution of the central compartment (l)

$\alpha\beta$ = Hybrid rate constant (h^{-1}).

t = Time following start of infusion (h).

T = Time of infusion (h).

The derivations of the pharmacokinetic model are shown in appendix I.

The bioavailability (F) was calculated according to the following equation:

$$F = \frac{AUC_{oral} \times Dose_{i.v.}}{AUC_{i.v.} \times Dose_{oral}}$$

2.4. CONCENTRATION-EFFECT RELATIONSHIP ANALYSIS

The establishment of a relationship between plasma drug concentrations and effect is an important tool in the assessment of dose requirements in individual patients.

Most previous studies on calcium antagonist drugs have shown a wide interindividual variability in pharmacokinetics and such variability clearly contributes to the large differences in response between patients.

Although, a simple direct relationship between plasma concentration and effect can be established for some drugs, for many drugs such a relationship cannot be simply established, primarily because the concentration-time profile is out of phase with effect-time profile (Whiting and Kelman, 1980) (figure 2.3.). This phase discrepancy might reflect the formation of an intermediate active

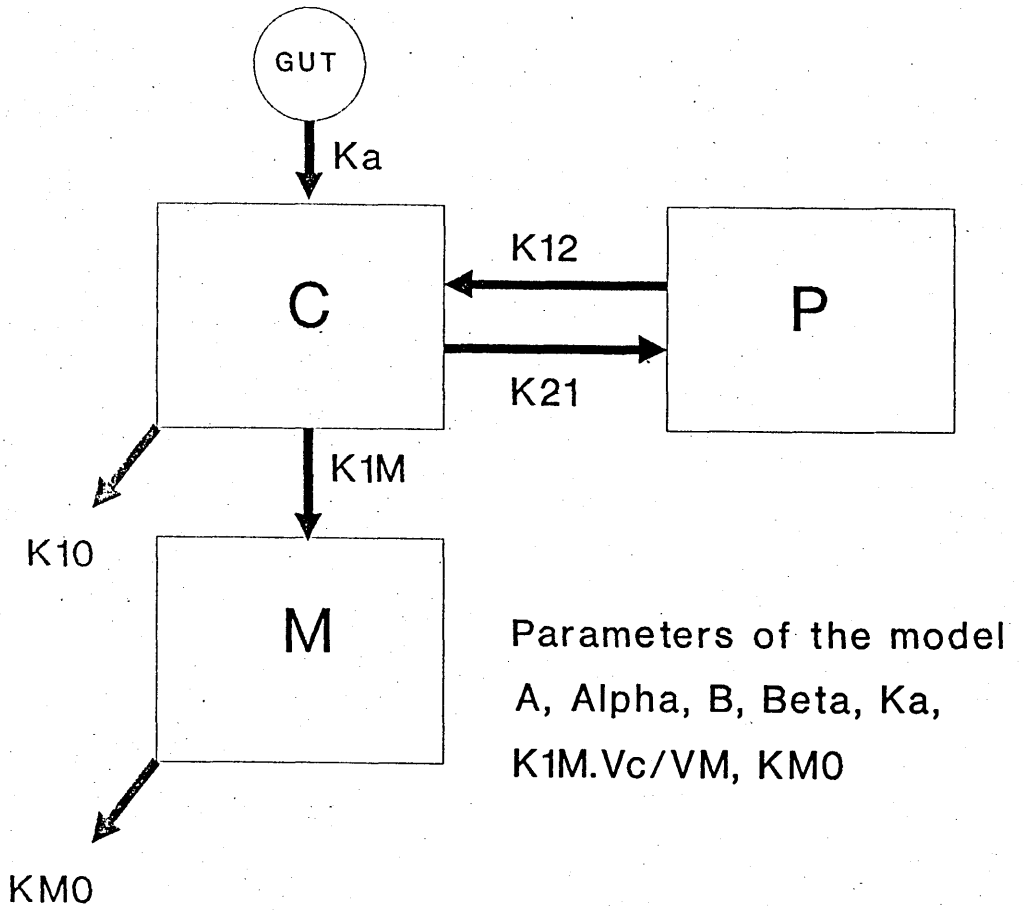


Figure 2.1. Schematic representation of two compartment model following oral administration
C=central compartment.
P=peripheral compartment.
K_{12}=first order rate constant of drug transfer between the central and peripheral compartment
K_{21}=first order rate constant of drug transfer between the peripheral and central compartment
K_{10}=elimination rate constant from the central compartment.
K_{1M}=first order rate constant of drug transfer between the central and metabolite compartment
K_{M0}=elimination rate constant from the metabolite compartment.

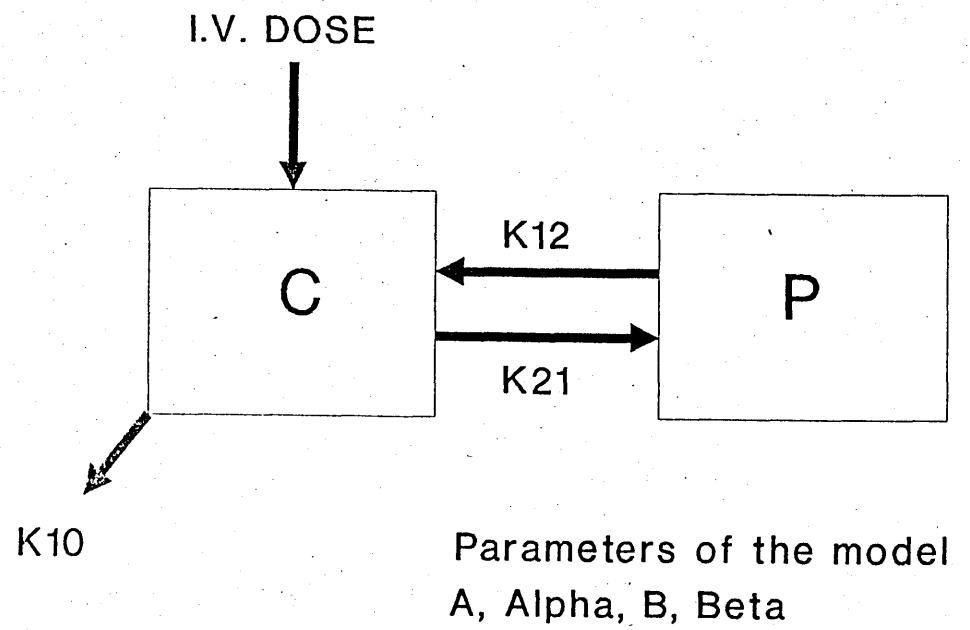


Figure 2.2. Schematic representation of two compartment model following intravenous administration
C=central compartment.
P=peripheral compartment.
K_{12}=first order rate constant of drug transfer between the central and peripheral compartment
K_{21}=first order rate constant of drug transfer between the peripheral and central compartment
K_{10}=elimination rate constant from the central compartment.

substance or metabolite or simply reflect the time required for drug to be made available at the receptor site and produce an effect. To take account of this delay in effect Sheiner et al., (1979) has developed a model which integrates kinetics and dynamics. This model augments the central and peripheral pharmacokinetic compartments with an effect compartment which is small enough that it feeds in only one direction from the predetermined kinetic compartments (figure 2.4.). The mathematical derivation of this compartment is:

$$dX_e/dt=K_{1e}X1 - K_{eq}X_e.$$

Where X1 is the amount of drug in the central compartment, X_e is the amount of drug in the effect compartment, K_{1e} and K_{eq} are first order rate constants which describe the entry and removal of the drug to and from the effect compartment. To characterise the concentration-effect relationship, effect (E) which is, for example, reduction in blood pressure is related to the concentration of drug in the effect compartment (C_e) by means of a Langmuir (E_{max}) model or a simple linear model of the form $E=mC_e + i$. In many instances, for the range of the concentrations and the magnitude of the effects obtained in clinical studies, a linear model is often appropriate (figure 2.5.). Thus, a concentration-effect relationship can be established for each individual patient and, from the linear model, parameters such as "sensitivity" ("m") can be obtained: this is defined as the magnitude of blood pressure reduction in mmHg per unit change in drug concentration.

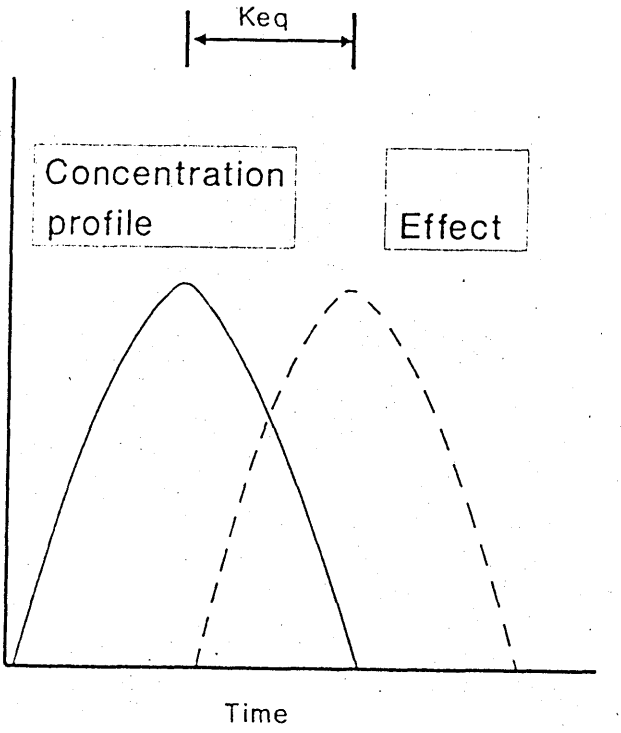


Figure 2.3. A schematic representation of the discrepancy between the concentration time profile and effect profile.

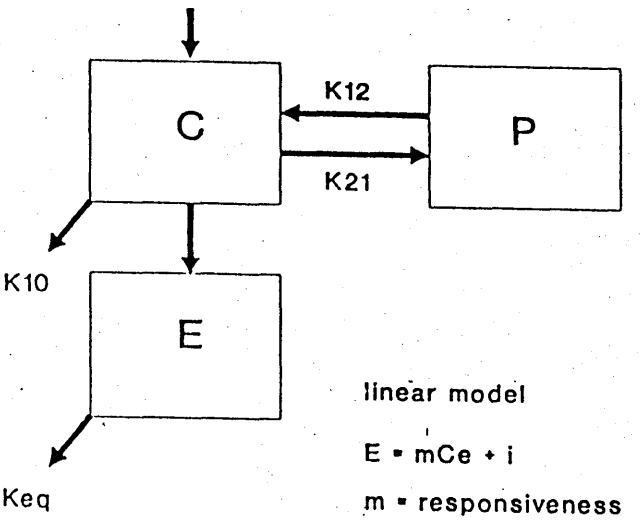


Figure 2.4. Extended kinetic model which incorporates the pharmacokinetic and pharmacodynamic model, C= central compartment, P= peripheral compartment, E= effect compartment which is small enough to effect the pharmacokinetic compartments.

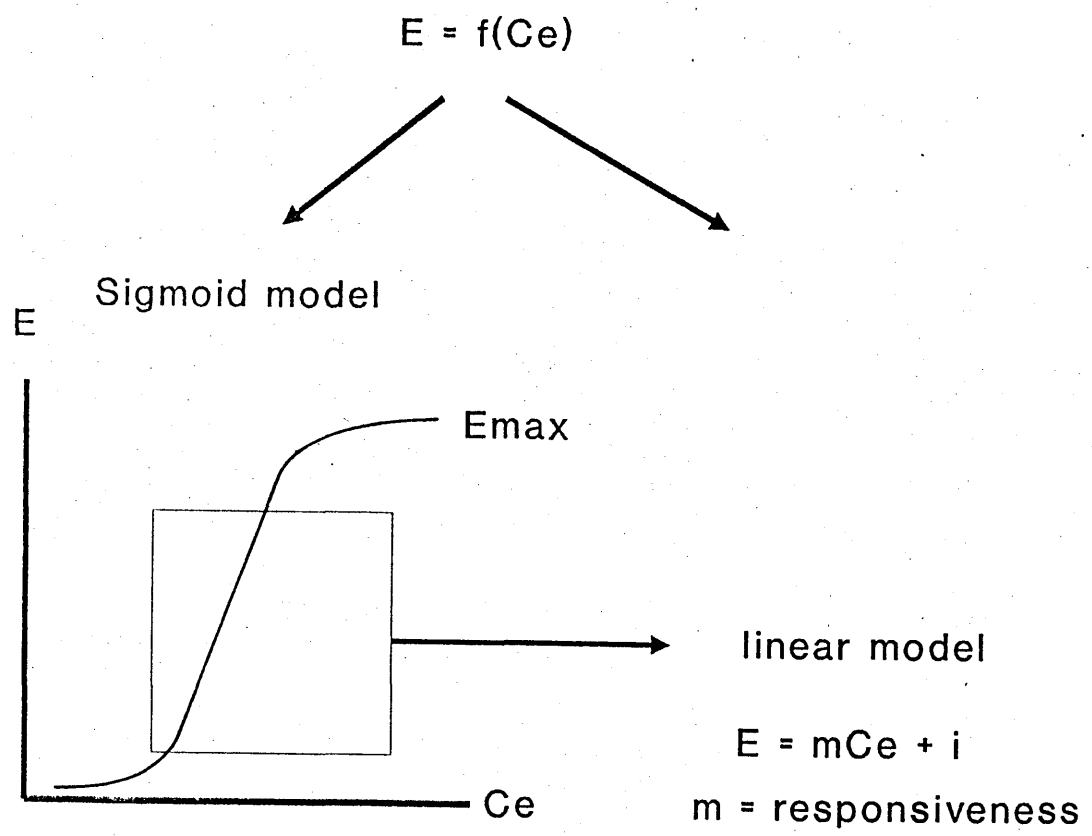
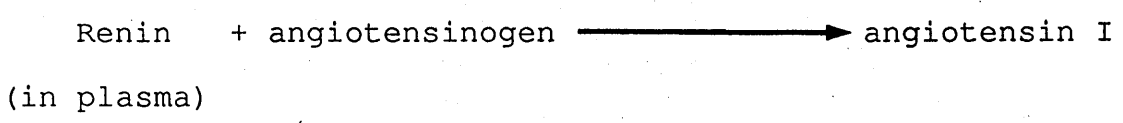


Figure 2.5. Effect (E) which is blood pressure reduction as a function of drug concentration in the effect compartment (C_e). A sigmoid relationship could well describe the data but in clinical practise the linear part of the sigmoid curve (marked by box) is often more relevant.

2.5. HORMONES AND METABOLIC ANALYSIS

2.5.1. PLASMA RENIN ACTIVITY

Plasma renin activity was measured by an indirect method which involves incubating plasma with the substrate, angiotensinogen (Derkx et al., 1972).



The enzymatic reaction which leads to the generation of angiotensin I is stopped after a fixed period and angiotensin I levels are determined by radio-immunoassay.

The normal range for plasma renin activity is 0-12 ng AI/ml/h with intra- and inter-assay coefficient of variation of 7% and 5.5% respectively.

2.5.2. PLASMA ALDOSTERONE

Plasma aldosterone concentration was measured by radio-immunoassay using commercially available kit methods (SB-ALDO-M, SB-ALDO, SB-ALDO-D). Venous blood was collected into lithium heparin tubes. The principle of the assay depends on competition between ^{125}I-labelled aldosterone and the aldosterone contained in standards or in specimens with a fixed and limited number of antibody binding sites. After incubation, the amount of labelled aldosterone bound to the antibody is inversely related to the amount of unlabelled aldosterone present in the sample. The method adopted for

bound/free separation is based on the use of antibody-coated tubes in which the antibody is fixed on to the tube walls by the method of Catts. Plasma samples, or standards, were added to the antibody treated tubes, mixed by vortex and incubated at 2-8 ^{o}C for 18-24 hours. The contents of the tubes were carefully aspirated and rinsed and the radioactivity (^{125}I) of the tubes was counted by a gamma counter. For each group of tubes, the mean count (corrected for background activity) was calculated as a percentage of the zero "standard" group:

$$B/Bo\% = \frac{\text{std or sample}}{\text{std "0"}} \times 100$$

Where B is the counted radioactivity, Bo is the background. Std "0" is the counted radioactivity of the radioactive free standard sample.

The standard curve was constructed by plotting B/Bo versus the concentrations (pg/ml) on linear-linear coordinates.

The assay is sensitive for concentrations greater than 10.5 $\pm$ 2.2 pg/ml. Within-assay coefficient of variation is 7.8% and between assay is 12.4%. Normal range is 12-125 pg/ml supine and 70-295 pg/ml erect.

2.5.3 SERUM LIPIDS

The serum was separated by centrifugation for 10

minutes at 1000 x g and stored at 4°C for not more than 5 days prior to the analysis. Serum triglyceride was measured enzymatically (Bucolo & David, 1973) using a Technicon RA 1000. Cholesterol concentrations were estimated manually using an enzymatic technique (Allain et al., 1974). Very low density lipoprotein (VLDL) was separated by ultracentrifugation (Airfuge. Beckman Instruments Ltd.) (Farish et al., 1983); High density lipoprotein (HDL) and subfractions were quantified using the method of Eyre et al., (1981) and Low density lipoprotein (LDL) cholesterol levels were calculated by subtraction.

Free fatty acid was analysed using a commercial kit (Wako Chemical GmbH, GDR).

2.5.4. SERUM INSULIN

Blood samples were collected in plain tubes. Serum was separated by centrifugating for 10 minutes at 1000 x g and stored at -20 °C until analysis.

The serum was diluted with assay diluent (0.05 m phosphate buffer, PH 7.4 containing bovine plasma albumin) and incubated overnight at 4 °C. 100 µl of ^{125}I-labelled insulin is added and the mixture is incubated for 3 hours at room temperature. 200 µl of sheep anti-guinea pig serum linked to sepharose CL-4B beads (solid-phase 2nd antibody) is added and the mixture then shaken for 30 minutes, separated by washing and centrifugated between washes with 2 ml of 0.9% normal saline using the Watson Marlow semiautomatic washing apparatus. Gamma counter NE 1600 linked to apple II

linked to apple II microprocessor was used to calculate the level of insulin.

2.5.5. PLASMA LACTATE

The lactate level was analysed by enzymatic UV-method (Boehringer Mannheim GmBH). The principle of the test is based on the conversion of L-lactate to pyruvate by the enzyme lactate dihydrogenase (LDH).

$$\text{L-Lactate} + \text{NAD} \rightleftharpoons \text{pyruvate} + \text{NADH} + \text{H}^+$$

$$\text{Pyruvate} + \text{L-glutamate} \rightleftharpoons \text{L-alanine} + \text{alpha-oxoglutarate}$$

2 mls of blood were collected in fluoride/EDTA-tubes and centrifuged for 5 minutes at 3000 rpm. Plasma was separated and stored at -20°C until analysis. Normal range is 5.7 - 22 mg/100 ml (0.63 - 2.44 mmol/l).

2.5.6. PLASMA ADRENALINE AND NORADRENALINE

Plasma adrenaline and noradrenaline concentrations were estimated by radio-enzymatic assay of Peuler and Johnson (1977). Venous blood samples were collected into lithium heparin tubes. Plasma was separated by centrifugation at 4°C and 300 rpm for 10 minutes and stored at -70 °C until analysis. The principle of the method is based on the utilisation of the enzyme catechol-O-methyl transferase (COMT) to transfer a radioactive methyl group from S-adenosyl-L-methionine (SAM) to an endogenous catecholamine

levels of picomoles. The intra-assay coefficient of variation for adrenaline and noradrenaline was 13% and 10% respectively.

2.5.7. THYROID FUNCTION TEST (TSH & T4)

Thyroxine (T4) was analysed using Amerlex-MT4 radioimmunoassay kit. The sensitivity of the methods is better than 4.0 nmol/l. The method is specific for thyroxine (T_4). However cross-reactivity occurs with L-tri-iodothyronine (12%). The normal range of T_4 = 62-165 nmol/l.

TSH was analysed using radio-immunoassay method using RIA-Gnost hTSH kit. In normal thyroid function TSH = 0.1 - 4 μIU/ml.

2.6. PHARMACODYNAMICS MEASUREMENTS

2.6.1. BLOOD PRESSURE AND HEART RATE

After at least 20 minutes in the recumbent position, blood pressure and heart rate were recorded in duplicate by semiautomatic recorder (Sentron, Bard Medical) both supine and after 2 and 5 minutes in erect position. The "Sentron" monitor is an accurate, noninvasive arterial blood pressure monitor which uses the oscillometric method of pressure determination. The oscillometric method makes use of the pressure pulsations which are induced into the cuff by the pulsatile motion of the artery wall while partially occluded by a compressive cuff. Systolic and diastolic pressure are determined by a microprocessor analysis of the pulsation

amplitudes which are measured and recorded at a succession of cuff pressure. Accuracy of cuff pressure measurement correlates with a pressure standard ±1% (Johnson et al., 1985).

2.6.2. DETERMINATION OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW.

Effective renal plasma flow and glomerular filtration rate were estimated from the clearance of [^{125}I]-hippuran and [^{51}Cr]-EDTA respectively. The radioactive compounds were administered simultaneously by intravenous bolus injection (5mls total volume). Blood samples were collected from the contralateral side before the injection and at 2, 5, 10, 20, 30, 60, 90, 120, 180, 240 minutes after the injection. Patients were asked to empty the urinary bladder before the injection and then urine was collected over the following 4 hours. Fluid intake was encouraged during the test.

Blood samples were centrifuged and plasma removed . Equal aliquots (usually 1 ml) were taken from each of the plasma samples, the urine and a standard solution consisting of the dose made up to one litre with water. The activity of the samples was measured by a Packard Counter Auto-Gamma, model 5650). The results obtained for one subject are presented in table (2.1.) and figure (2.6.).

TABLE 2.1.

THE RADIOACTIVITY COUNT OF ^{51}Cr-EDTA FOR A REPRESENTATIVE PATIENT (J.M.) OBTAINED FROM GAMMA COUNTER

Time (min)	**Count**	**Background corrected activity**	**plasma activity * (% dose/l)**
Bkg	48		
0	51		
2	986	935	14.62
5	586	535	8.36
10	456	405	6.33
20	384	333	5.20
30	344	293	4.58
60	272	221	3.45
90	233	182	2.84
120	214	163	2.55
180	173	122	1.90
240	141	90	1.40

* Plasma activity =(plasma count-BKgd)/(std.count-BKgd)x100

Standard count =6442

Urine count =16114

Total urine volume/4 hours=245 mls

The calculated area under the curve (by trapezoidal rule) of the plasma activity expressed as % dose/l=715

$$\% \text{ dose excreted in urine} = \frac{(16114-48)\times 245 \times 100}{(6442-48)\times 1000}$$

$$= 61.56\%$$

Clearance (GFR) = dose/AUC = 61.56/715 =86 ml/min.

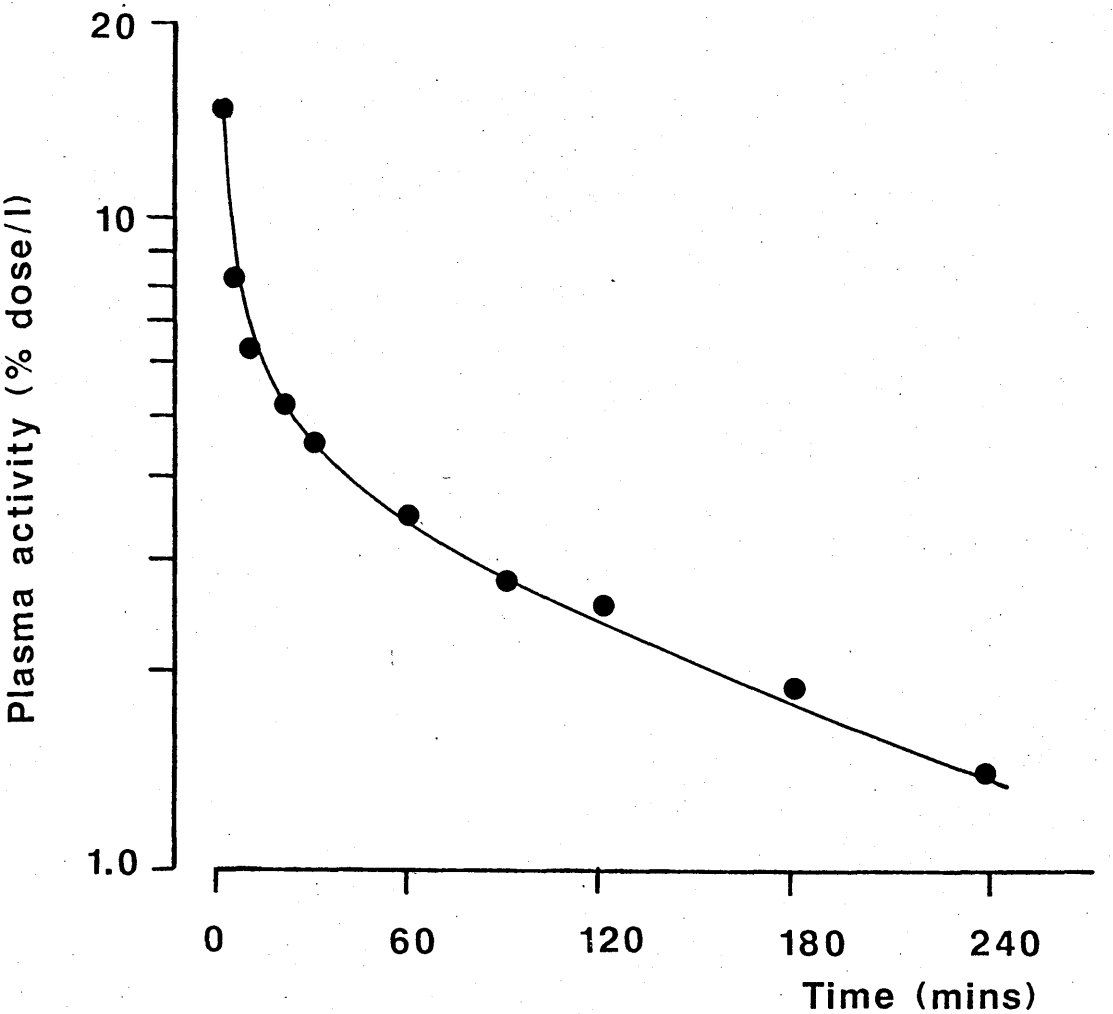


Figure 2.6. A graph of the plasma activity of ^{51}Cr-EDTA (for GFR measurement) vs time for a representative subject. The area under the concentration time curve (AUC) was calculated by trapezoidal role. Elimination rate constant was calculated by fitting the terminal part of the curve to one exponential.

If urine collection is not complete or there is no urine data then it is assumed that 100% of the radioactive material injected is excreted in the urine after an infinite time. Hence GFR=100/$AUC_{0-\infty}$. The area under the curve to infinity is calculated by adding the AUC_{0-4} to the AUC of the terminal part which can be obtained by fitting the terminal part of the elimination phase of the plasma activity to an exponential function and then by dividing the last concentration by the elimination rate constant. Hence GFR (extrapolated) = 100 x 1000/1016 = 98 ml/min.

The determination of renal plasma flow is similar to the GFR except that 5% of ^{51}Cr-EDTA count subtracted from the radioactivity count of [^{125}I]-hippuran to allow for the interference caused by ^{51}Cr-EDTA.

The individual data for glomerular filtration rate or renal plasma flow are adjusted for body surface area. The average normal body surface area for an adult is 1.73 square meters. Body surface areas for individuals were calculated from an equation described in Martindale (Reynolds, 1982).

$$S = W^{0.45} \times H^{0.725} \times 71.84 \text{ (a constant)}$$

Where S = surface area in square centimetres, W = weight in kilograms, and H = height in centimetres.

2.6.3. DETERMINATION OF LIVER BLOOD FLOW

Indocyanine green (ICG) was administered intravenously as a bolus dose of 0.5 mg/kg and blood samples were

collected every 3 minutes over 24 minutes. The concentrations of ICG in plasma were analysed by HPLC method (Rappaport et al., 1982). The concentration time profile for each individual and after each treatment was fitted to a one compartment model. Volume of distribution and plasma clearance of ICG were calculated directly from curve fitting (figure 2.7.). The plasma clearance of ICG which approximates to liver plasma flow is corrected for haematocrit to calculate apparent liver blood flow (Caesar et al., 1961).

$$\text{Liver blood flow} = \frac{\text{Clp} \times 100}{1 - \text{HCT\%}}$$

2.6.4. LEFT VENTRICULAR EJECTION FRACTION

Radionuclide angiography has been increasingly utilised in the evaluation of left ventricular function in a variety of disease states. It is relatively safe and non-invasive and can be repeated on the same individual after a short period of time (for example 30 minutes).

The study was performed in the Nuclear Medicine Department, Stobhill general hospital.

The patients were injected with intravenous "Amerscan" (a stannous red blood cell agent containing stannous fluoride and sodium medronate) to facilitate the binding of the radioactive material to red blood cells. ECG electrodes were placed on the left and right shoulder and at the apex

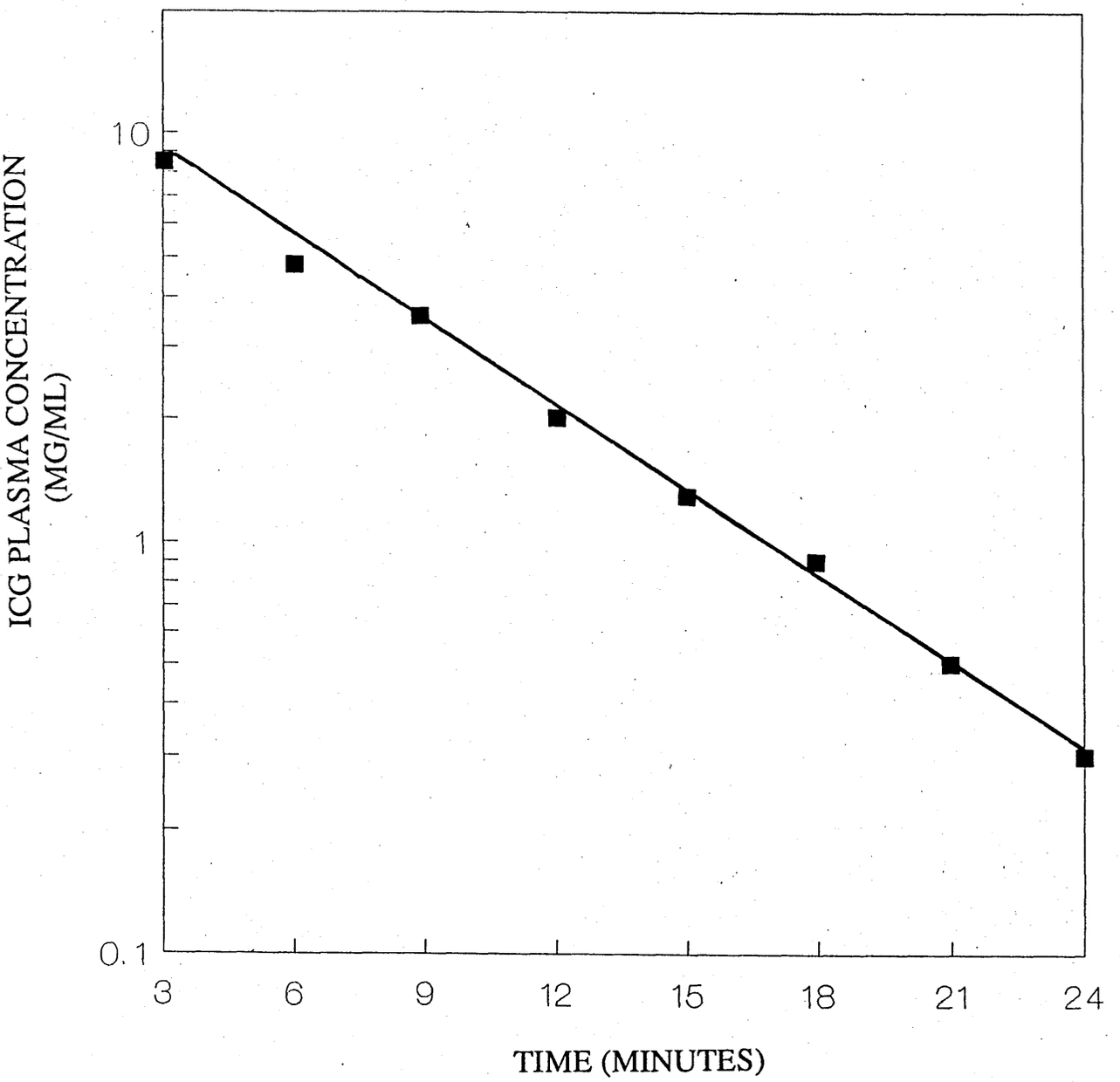


Figure 2.7. The concentration time profile of indocyanine green (ICG) injected as a bolus intravenous dose (0.5 mg/kg). One compartment model was appropriate for describing the concentration time profile.

of the heart. The electrodes were connected to a Rigel monitor and gating circuit which provides a suitable signal for input to a Nodecrest V77-600 computer (figure 2.8.).

The patients then rested in the supine position. Twenty to thirty minutes after the injection of the Amerscan, 800 MBq (22 mCi) ^{99m}Tc-pertechnetate was injected intravenously. This labels the circulating red blood cells with ^{99m}Tc. After a few minutes the face of the gamma camera was set at 45 to the horizontal and placed as close to the left side of the chest as possible.

A total of 4 X 10^6 counts were acquired by the gamma camera and computer. The computer divides the cardiac cycle (between two R waves) into 20 frames which show the radioactivity at different stages of the cardiac cycle. To ensure that a given frame represents the same stage of the cardiac cycle, beats which differ in length by more than 10% from the value specified at the start of the acquisition were rejected.

At the end of the acquisition, a region of interest (ROI) is drawn around the left ventricle on the end of diastole frame (usually frame 1). In addition a crescent shaped area is drawn around the outer wall of the left ventricle, to represent background activity. The left ventricle ROI is then corrected for background and the left ventricle activity-time curve is generated (fig 2.9.).

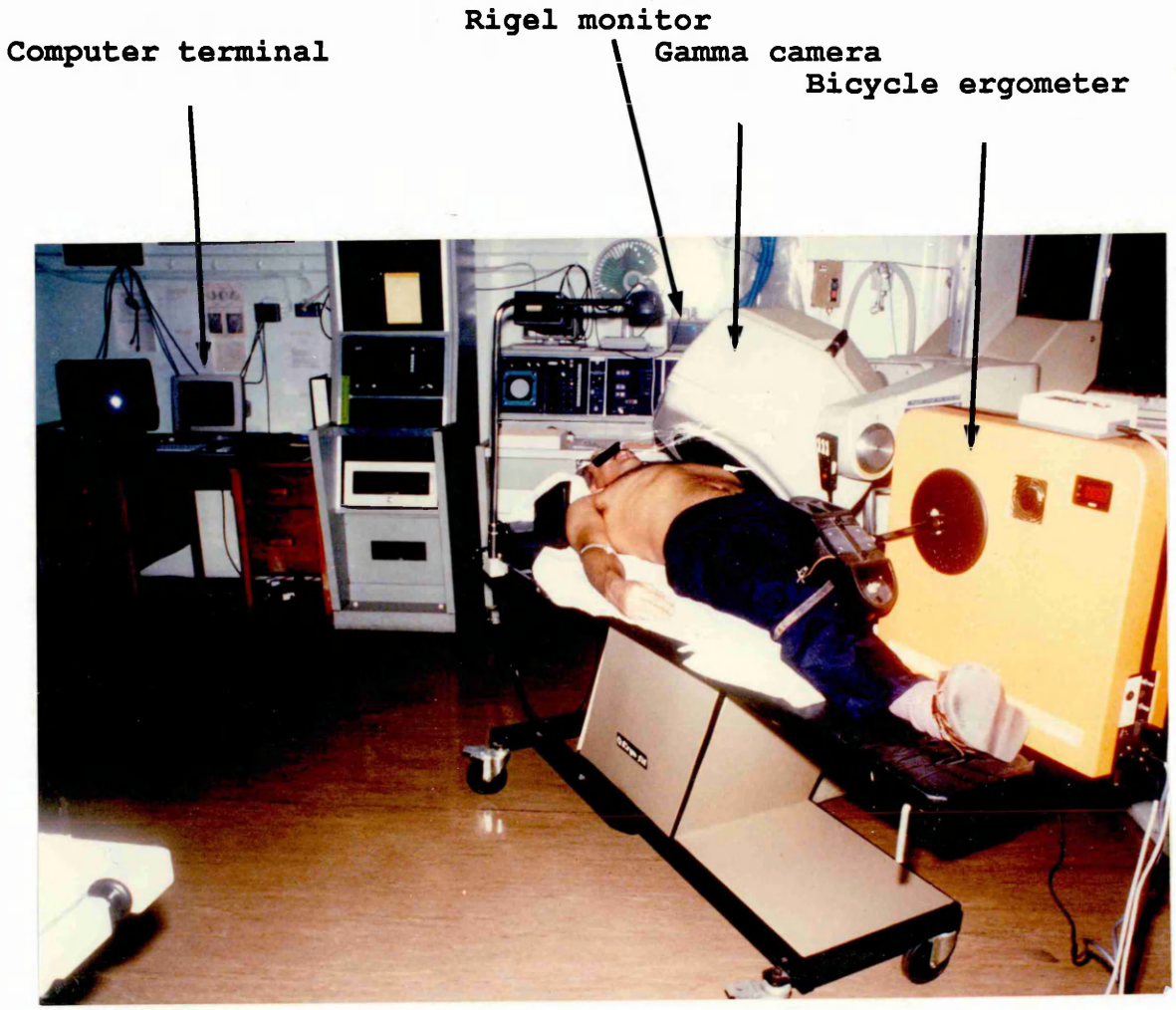


Figure 2.8. A photographic representation of the equipments used for the measurement of left ventricular ejection fraction at rest and during exercise:the face of the gamma camera placed close to the left side of the chest and linked to Nodecrest V77-600 computer. ECG electrodes were placed on the left side of chest and connected to Rigel monitor.

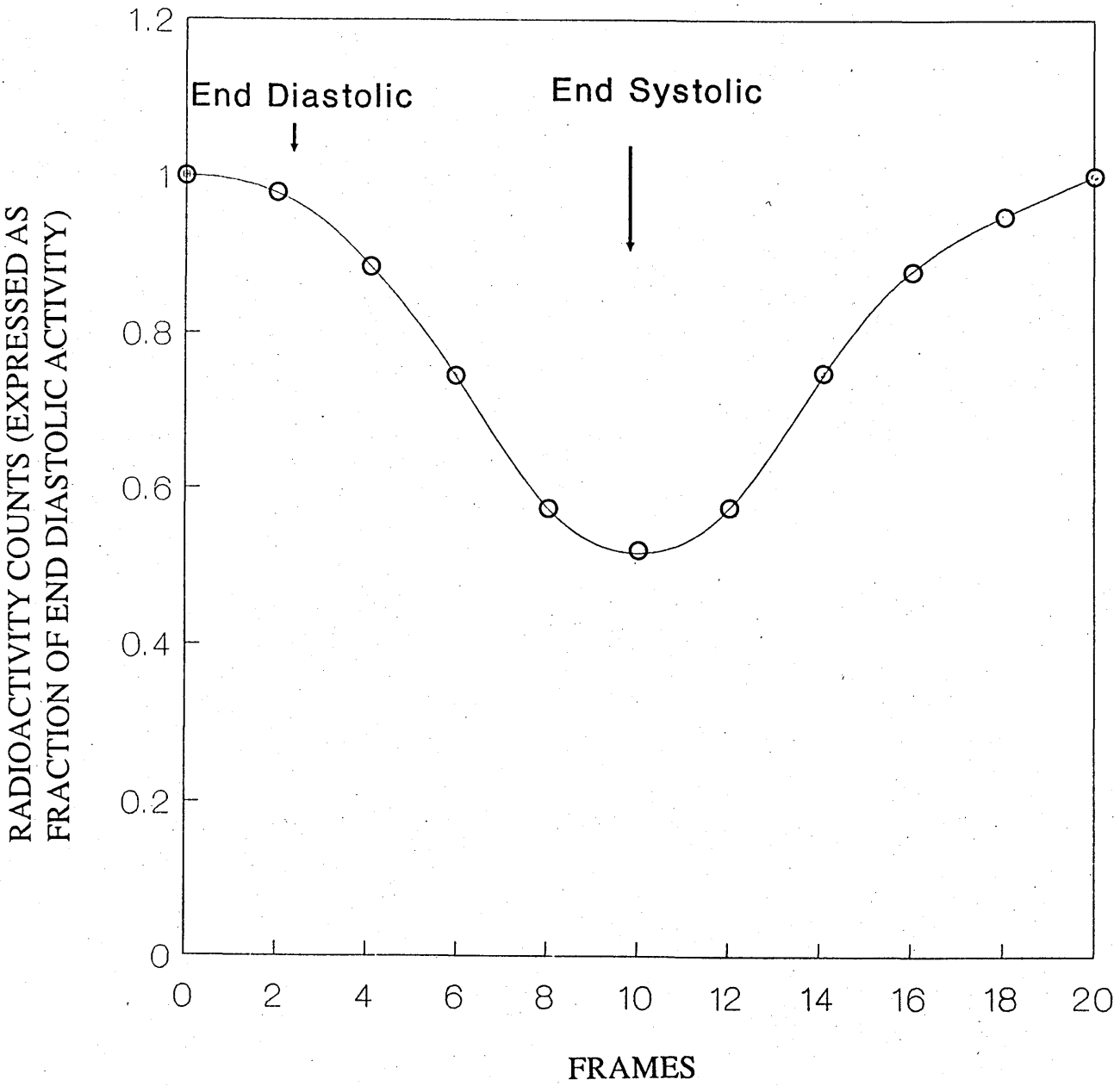


Figure 2.9. Left ventricle activity-time curve (expressed as fraction of end diastolic activity).

The minimum of this curve represents the point of end systole (ES) and the ejection fraction is calculated as:

$$\text{Ejection fraction \%} = \frac{ED - ES}{ED}$$

where ED = end diastolic radioactivity count

ES = end systolic radioactivity count

The cardiac cycle is divided into 20 frames; each frame takes 1/20 of a second (for a heart rate of 60 bpm). The mean difference in ejection fraction between observers is about 1% and the mean within subject difference is about 5%.

2.6.5. P-R INTERVAL

P-R interval is an electrocardiographic (ECG) term which refers to the distance on the ECG trace between the beginning of P-wave and beginning of Q-wave (figure 2.10.).

Lead II ECG was recorded at a speed of 50 mm/s and ten consequent cardiac beats were used for the calculation of P-R interval.

A digitiser linked to Nodecrest V77-600 computer was used for the calculation of P-R interval. This method is faster and relatively more accurate than using the ruler for the measurements.

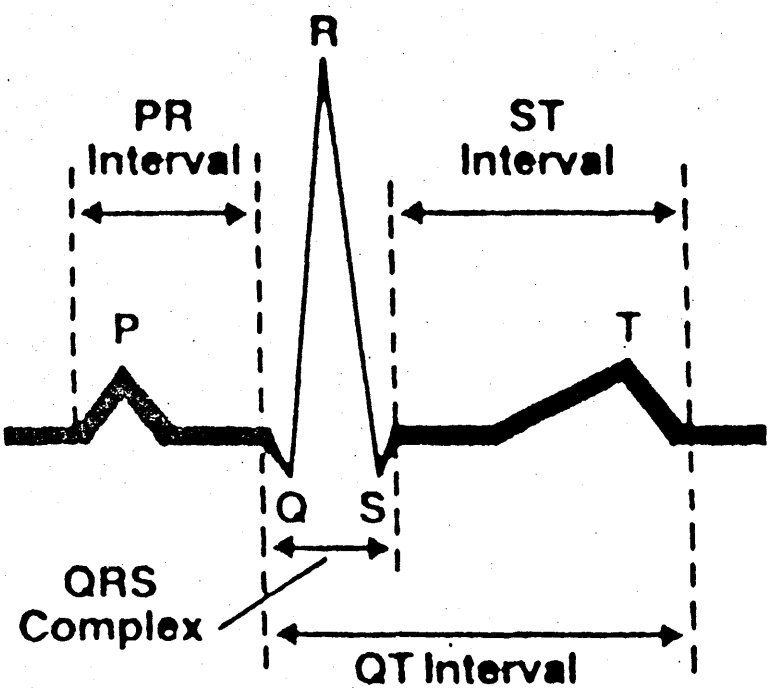


Figure 2.10. Schematic presentation of Electrocardiogram (ECG). PR interval measured from the beginning of P wave to the beginning of Q wave.

Standard times (ms):

PR = 120 - 200
ST = 270 - 330
QT = 350 - 420
QRS = 80 - 110

2.7. DATA ACQUISITION AND STATISTICAL ANALYSIS

Data were transcribed and stored on an electronic spread sheet (Lotus 123) which offered the following advantages:

1. Data can be manipulated with reasonable flexibility so that full exploration is relatively straightforward and an output file, to suit the format needed by a particular statistical package, can easily be prepared.

2. The output data file can be exported to powerful statistical packages such as Statgraphics or Minitab which run on microcomputers.

3. The output file can be also transferred to the University main frame computer using a transfer programme (Kermit, Ver 2.31) which is available in the Department on Apricot personal computer. Transferred data can be analysed directly by "Rummage" or "BMDP" statistical packages.

STATISTICAL ANALYSIS

The data were first plotted on appropriate graph paper and normality of distribution was tested using X^2 method. If the data did not follow the normal distribution pattern, various transformation were attempted, such as Logarithmic or natural Logarithmic transformation.

Parametric tests, which are potentially more powerful and more informative than non parametric tests, were preferred if possible, after appropriate data transformation, rather than application of non-parametric test.

Repeated measures analysis of variance (ANOVA) was used (using Rummage statistical package) for the evaluation of time and treatment effects for the repeated measurements of blood pressures and heart rate. This package has the advantage on "BMDP" of performing the test of time/treatment interactions.

For the analysis of the metabolic and hormonal responses to nicardipine (chapter 8), because some of the repeated observations were interdependent and therefore potentially "correlated" (a critical violation of the assumption for regular ANOVA) (O'Brien et al., 1985), an alternative statistical test, multivariate analysis of variance (MANOVA), was considered. MANOVA is a generalisation of ANOVA that allows the researcher to analyse more than one dependent variable (Bray et al., 1985) but, if the sample size is small, it is less powerful and has no advantage over ANOVA.

Ninety five percentage (95%) confidence intervals were calculated based on the equation published by Altman and Gardner (1988). Power of the test to detect a given difference was calculated according to formulae described by Jacob Cohen (1979) and Machin & Campbell, (1987).

A P-value of less than 0.05 is considered statistically significant.

Bonferroni correction has been applied, as appropriate, to allow for multiple comparisons.

Various additional statistical tests, including paired and unpaired t-tests and linear regression analysis were

carried out for specific circumstances and are described in the appropriate chapters.

CHAPTER THREE

AGE AND ANTIHYPERTENSIVE EFFICACY OF VERAPAMIL: APPLICATION OF CONCENTRATION EFFECT ANALYSIS

INTRODUCTION

Buhler and colleagues (1982) have described a relatively greater antihypertensive efficacy to calcium antagonist drugs in elderly hypertensive patients and in patients with low plasma renin activity (Muller et al., 1984; Kiowski et al., 1985). Although the underlying mechanism was not clearly defined there are two fundamental possibilities: an age related increase in plasma drug levels, presumably reflecting a reduction in plasma drug clearance; or an increase in tissue responsiveness; or possibly both.

Recent studies have demonstrated that it is possible to define a relationship between the plasma concentrations of different antihypertensive drugs and the blood pressure reductions in individual patients (Meredith et al., 1983; Elliott et al., 1984; Donnelly et al., 1988) by means of concentration-effect analysis or "effect modelling" , as first proposed by Sheiner et al. (1979). This approach, which integrates the kinetic and dynamic data, has provided a useful tool for investigating antihypertensive responses. The aim of this study, therefore, was to investigate the effect of age on the pharmacokinetics and pharmacodynamics following single and multiple doses of verapamil by application of an integrated concentration-effect modelling technique.

SUBJECTS AND METHODS

Fourteen patients with mild to moderate essential

hypertension (Four male, 10 females, age 41-79 years, weight 50-92 kgs) (table 3.1.) were studied. All previous antihypertensive medication had been withdrawn at least one month prior to the study. The protocol was approved by the hospital ethical committee and all patients gave informed consent. The study design was single blind (patient-blind), randomised and crossover. The patients received placebo for 2 weeks followed by verapamil 120 mg twice daily for 4 weeks and attended for three 8-hour study days to evaluate the effect of placebo, first dose (120 mg) verapamil and steady state verapamil (120 mg). On each study day, blood pressure and heart rate (supine and erect) were measured by semiautomatic recorder (Sentron; Bard Medical). Blood samples were withdrawn at frequent intervals for 8 h after dosing for the determination of plasma concentrations of verapamil and norverapamil according to the method of Cole et al. (1981). In 8 "elderly" patients (patients no. 1,2,5,6,9,10, 13,14) (age range 63-79 years) apparent liver blood flow, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured. Apparent liver blood flow was calculated from the clearance of intravenously administered indocyanine green (0.5 mg/kg) and GFR/ERPF were measured by standard radioisotope methods. These test substances were simultaneously administered one hour after drug administration.

TABLE 3.1.

THE CLINICAL CHARACTERISTICS OF PATIENTS

Subject	Age (years)	Weight (kg)	Pretreatment B.P. (mmHg)	PRA (ng AI/ml/h)
1	69	74	184	0.86
2	72	76	174	1.32
3	64	77	182	1.68
4	41	76	185	1.93
5	70	50	177	1.76
6	77	60	216	1.43
7	50	52	172	1.46
8	51	79	197	1.19
9	78	63	231	1.68
10	79	58	171	2.13
11	58	63	192	1.28
12	56	92	159	1.89
13	77	58	200	2.13
14	63	57	203	2.24
Mean	64.6	66.8	189	1.64
Sd	12.0	12.2	19	0.40

PRA : Plasma Renin Activity

The pharmacokinetics of verapamil and norverapamil were most appropriately described using an integrated model with two compartments for drug disposition and a third compartment for the metabolite. Concentration-effect analysis was applied to integrate the pharmacokinetic and pharmacodynamic profiles. The effect profile (erect systolic or erect diastolic blood pressure corrected for placebo response) was fitted to a hierarchy of models to account for effects from drug alone and from metabolite. In all patients, following both acute and chronic treatment, the model accounting for effect from drug alone was most appropriate (see methods chapter) and there was a linear relationship between drug concentration (in the effect compartment) and the measured effect. Two parameters were derived from this approach; "m", which is an index of "responsiveness" i.e. the change in blood pressure per unit change in drug concentration in the effect compartment and "k_{eq}" a first-order rate constant characterising the temporal discrepancy between the concentration and effect profiles.

The results throughout are presented as mean $\pm$ sd and comparison of the pharmacokinetic results was by Student's t-test (paired data).

RESULTS

Pharmacokinetics

The apparent oral clearance was significantly reduced

at steady state to 1.4 ± 1.0 compared with 2.8 ± 1.4 l/min with acute dosing. This was associated with a significant prolongation of terminal elimination half life from 3.8 ± 1.2 to 11.2 ± 5.8 h with chronic dosing.

Peak plasma concentration of verapamil was increased with chronic dosing from 202 ± 104 to 263 ± 104 ng/ml but this was not statistically significant. The ratio of the AUC norverapamil to the AUC of verapamil was increased with chronic dosing from 0.8 ± 0.2 to 1.0 ± 0.2 but this was not significant; P = 0.0502, 95% C.I.= -0.3,0.0002 (table 3.2.).

Liver and renal function

Apparent liver blood flow was higher both with the first dose and with chronic dosing of verapamil, to 1.670 ± 0.700 and 1.640 ± 0.630 l/min respectively, but these were not statistically different from 1.200 ± 0.380 l/min with placebo (table 3.3.). Acute and chronic dosing of verapamil had no effect on glomerular filtration rate or effective renal plasma flow (table 3.4.).

Pharmacodynamics

There were significant falls in supine blood pressure and erect blood pressure following acute and chronic administration of verapamil. For example, the erect blood pressure (after 5 minutes standing), expressed as an average of all recordings obtained over the 8-hours, was reduced from 184/102 mmHg with placebo to 165/92 mmHg with acute therapy and to 154/85 mmHg with chronic treatment.

TABLE 3.2.

THE PHARMACOKINETIC OF VERAPAMIL

Subject	Cl/F (l/min)		Apparent elimination half life (h)		Cmax (ng/ml)		AUC Ratio *	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
1	5.7	2.3	3.5	7.6	98	142	1.1	1.3
2	5.8	3.0	3.0	4.0	104	133	0.8	0.7
3	2.6	0.7	6.0	8.7	131	398	0.5	0.9
4	2.3	0.8	3.4	22.3	234	289	0.8	0.9
5	1.3	0.6	4.1	11.8	443	323	0.9	1.1
6	3.2	4.1	2.3	4.3	181	125	0.7	1.1
7	2.0	1.4	5.5	15.8	228	323	0.6	0.7
8	2.9	0.7	4.5	10.9	134	402	1.3	1.0
9	2.3	0.9	3.9	13.7	183	217	0.7	1.1
10	2.4	0.9	4.7	8.2	225	392	0.9	1.1
11	3.3	1.3	1.9	6.6	99	118	1.0	1.0
12	1.1	0.9	3.9	19.3	264	287	0.8	1.1
13	3.3	0.9	2.0	6.0	131	217	0.8	0.7
14	1.3	0.9	4.4	18.3	376	313	0.5	0.9
Mean	2.8	1.4	3.8	11.2	202	263	0.8	1.0
Sd	1.4	1.0	1.2	5.8	104	104	0.2	0.2
P value	0.01		0.0001		0.1		0.0502	
95% C.I.	0.46,2.4		-10.8,-4		-141,20		-0.3,0.0002	

* AUC Ratio refers to AUC of norverapamil/AUC verapamil.

P value refers to the results of paired t-test between acute and chronic.

95% C.I. is the 95% confidence interval for the mean difference between acute and chronic data.

TABLE 3.3.

APPARENT LIVER BLOOD FLOW (l/min)
MEASURED IN 8 ELDERLY HYPERTENSIVE PATIENTS

Patients no.	Placebo	Acute	Chronic
1	1.648	2.406	2.605
2	0.923	1.269	0.749
5	0.914	1.959	1.994
6	1.630	2.978	1.956
9	0.728	0.979	1.830
10	1.616	1.484	1.844
13	1.248	1.338	0.859
14	0.913	0.876	1.267
Mean	1.200	1.661	1.640
Sd	0.380	0.731	0.630

TABLE 3.4.

RENAL FUNCTION MEASURED IN 8 ELDERLY HYPERTENSIVE PATIENTS

a. Glomerular filtration rate (ml/min)

Patients no.	Placebo	Acute	Chronic
1	77	77	74
2	75	79	75
5	53	66	61
6	87	89	80
9	64	73	67
10	32	30	32
13	64	59	52
14	58	41	43
Mean	63.8	64.3	60.5
Sd	16.9	20.0	16.9

b. Effective Renal Plasma Flow (ml/min)

Patients no.	Placebo	Acute	Chronic
1	268	284	274
2	285	383	324
5	254	288	286
6	360	416	408
9	268	315	276
10	164	162	160
13	231	235	214
14	264	167	187
Mean	261.8	281.3	266.1
Sd	54.6	91.9	79.5

The data are corrected for 1.73 m^2 body surface area.

Concentration-effect analysis

Responsiveness to acute and chronic verapamil administration is presented in table 3.5. On average, there was a reduction of 0.13 mmHg/ng/ml in systolic and of 0.063 mmHg/ng/ml in diastolic blood pressure following the first dose. There were highly significant correlations between responsiveness to acute dosing of verapamil and initial blood pressure ($r = 0.79$; $P<0.001$) and between chronic dosing and initial blood pressure ($r = 0.7$; $P<0.005$; figure 3.1.).

Similar results were obtained for erect diastolic blood pressure and again there were highly significant correlations between responsiveness to acute or chronic dosing of verapamil and initial blood pressure ($r = 0.77$; $P<0.001$; figure 3.2.).

When the responsiveness to the first dose of verapamil was correlated with the responsiveness after 4 weeks of treatment with verapamil, there were close correlations for both erect systolic ($r = 0.89$; $p = 0.0001$) and erect diastolic blood pressure ($r = 0.96$; $p = 0.0001$) with a slope approaching 1 and an intercept not significantly different from zero (Figure 3.3.). There was no significant correlation between responsiveness, for the fall in either erect systolic or in erect diastolic blood pressure, and age ($r = 0.17$; $p = 0.57$) or plasma renin activity PRA ($r = 0.22$; $p = 0.45$) (figure 3.4.).

TABLE 3.5.

RESPONSIVENESS (mmHg/ng/ml) TO ACUTE AND CHRONIC VERAPAMIL CALCULATED IN TERMS OF THE PLACEBO-SUBTRACTED CHANGES IN ERECT SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

Subject	Systolic B.P.		Diastolic B.P.	
	Acute	Chronic	Acute	Chronic
1	-0.177	-0.137	-0.083	-0.072
2	-0.149	-0.147	-0.064	-0.061
3	-0.080	-0.053	-0.031	-0.029
4	-0.164	-0.151	-0.084	-0.078
5	-0.107	-0.068	-0.054	-0.036
6	-0.214	-0.262	-0.103	-0.119
7	-0.112	-0.124	-0.048	-0.055
8	-0.129	-0.119	-0.078	-0.064
9	-0.267	-0.205	-0.129	-0.133
10	-0.055	-0.052	-0.038	-0.029
11	-0.090	-0.081	-0.042	-0.040
12	-0.053	-0.073	-0.026	-0.032
13	-0.116	-0.102	-0.049	-0.042
14	-0.156	-0.152	-0.061	-0.058
Mean	-0.133	-0.123	-0.063	-0.060
Sd	0.059	0.059	0.029	0.032

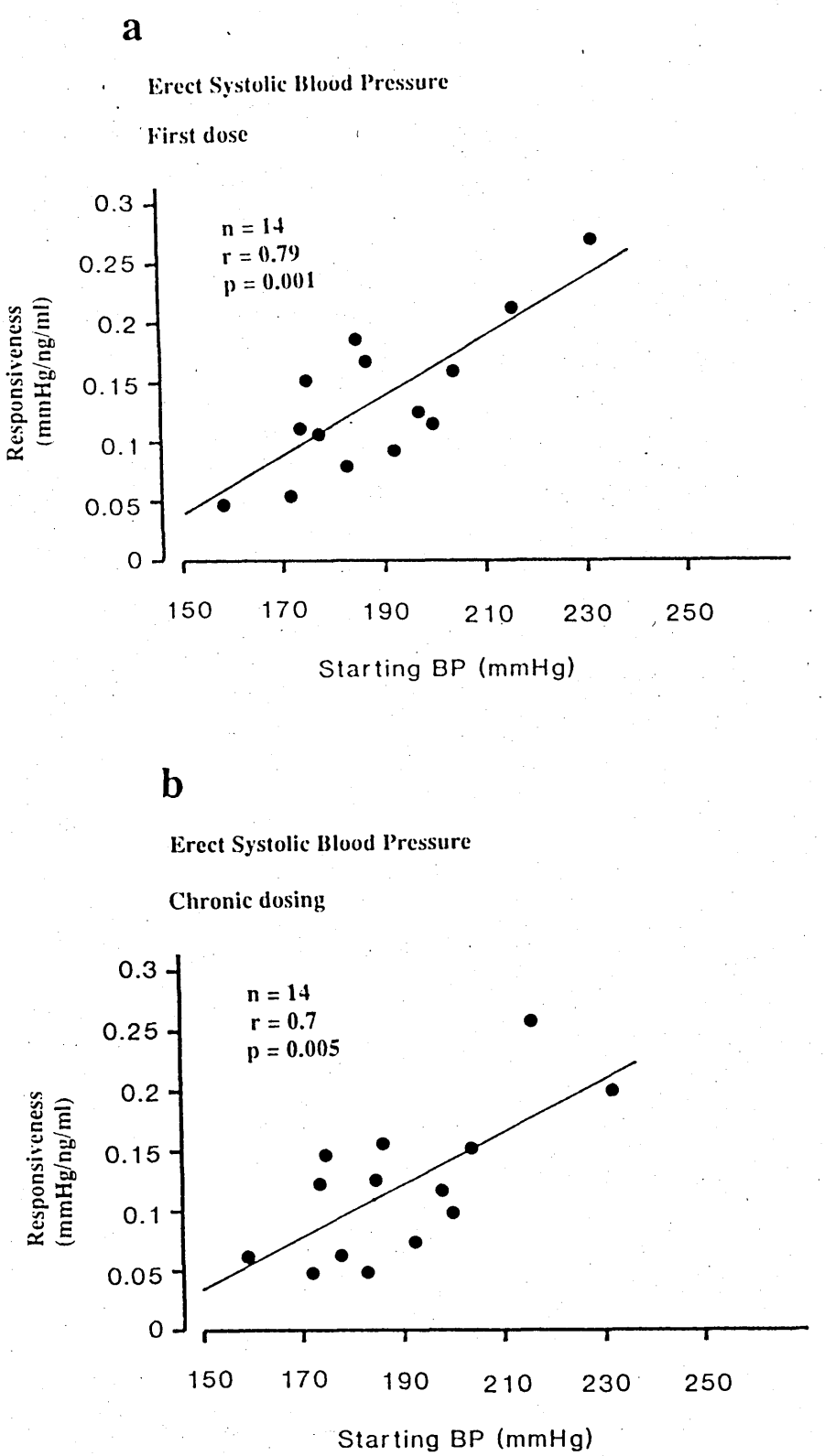


Figure 3.1. The correlation of responsiveness to verapamil (fall in erect systolic blood pressure, placebo corrected per unit concentration of drug) with pretreatment blood pressure after the first dose (a) and chronic administration (b) of verapamil. Power of correlation coeff.=98%

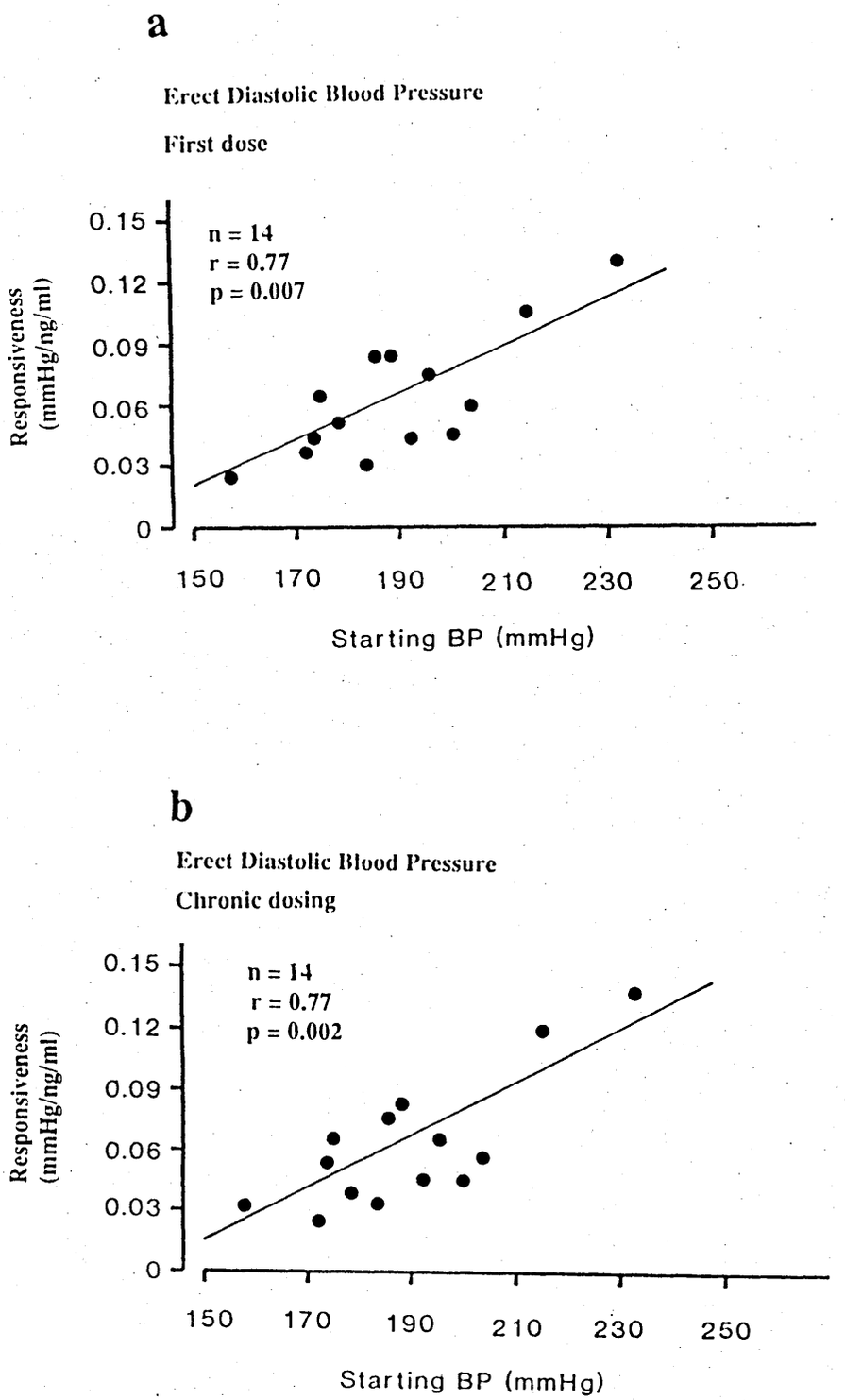


Figure 3.2. The correlation of responsiveness to verapamil (fall in erect diastolic blood pressure, placebo corrected per unit concentration of drug) with pretreatment blood pressure after acute (a) and chronic administration (b) of verapamil.

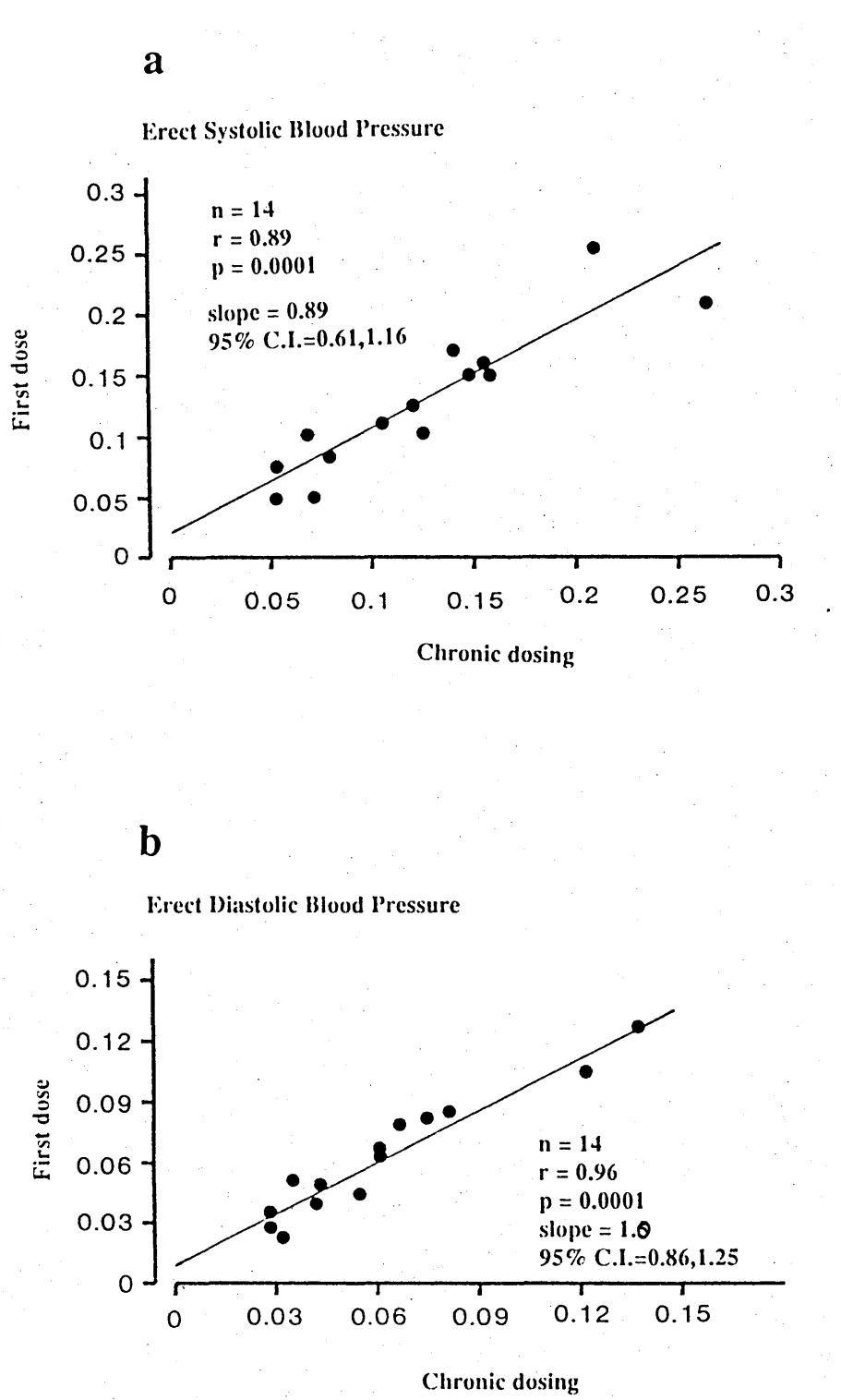


Figure 3.3. The correlation between the responsiveness to the first dose of verapamil and the responsiveness after 4 weeks of treatment for erect systolic blood pressure (a) and erect diastolic blood pressure (b).

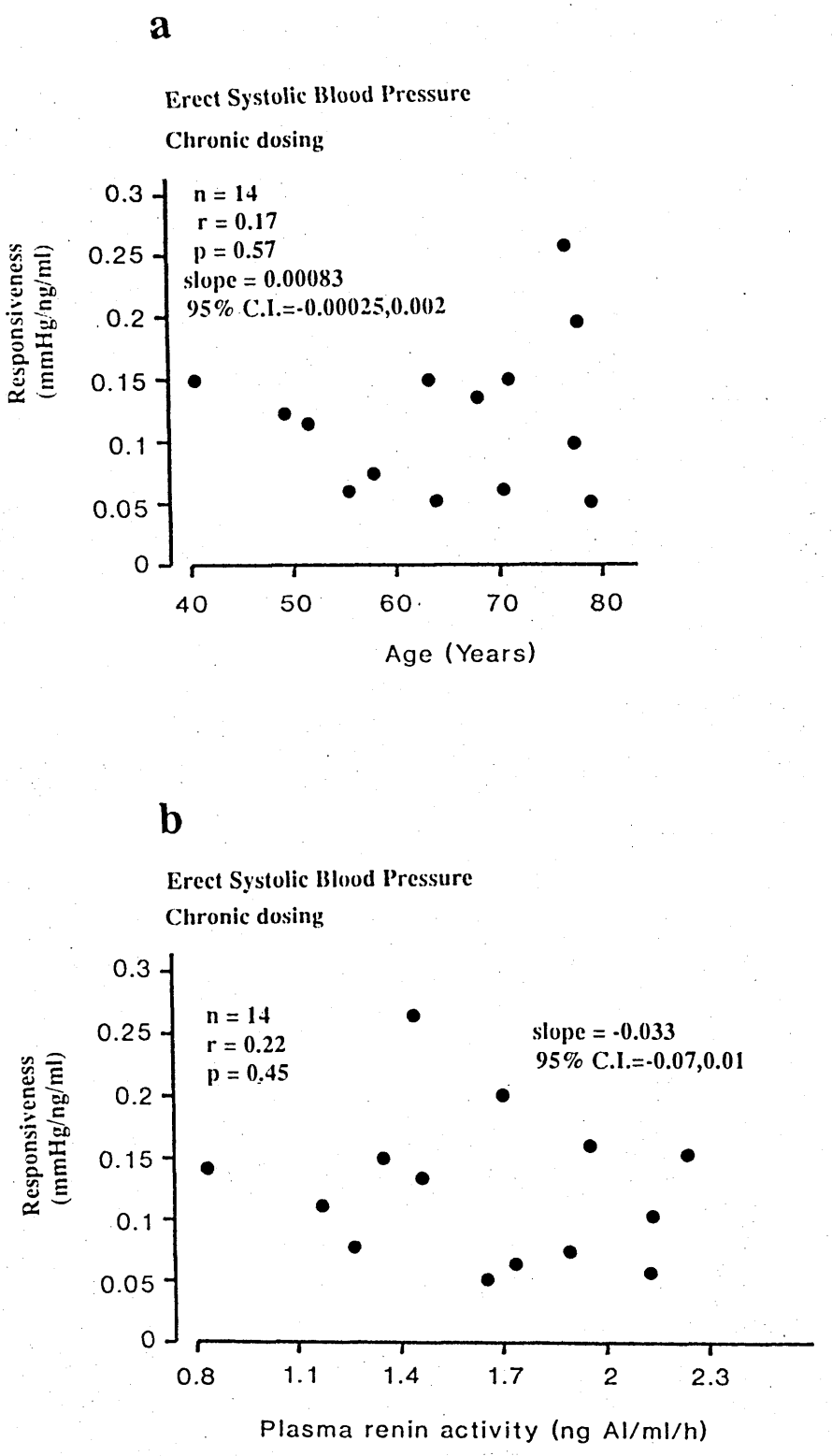


Figure 3.4. The correlation of responsiveness to verapamil (fall in erect systolic blood pressure at steady state, placebo corrected per unit concentration of drug) with age (a) or plasma renin activity (b).

DISCUSSION

In their evaluations of the antihypertensive efficacy of calcium antagonist drugs, some previous authors have correlated the maximum reduction in blood pressure with the height of the pretreatment blood pressure, or with age, or with plasma renin activity (Buhler et al., 1982; Erne et al., 1983). This approach not only has some statistical problems especially for comparing changes in blood pressure vs starting blood pressure since essentially the same variable appears on both axes (Gill et al., 1985) but it also ignores the inter-individual variability in the pharmacokinetics of the drug and considers the blood pressure response only at a single time point. Concentration effect modelling, which takes account of the variability in pharmacokinetics and which assesses the pharmacodynamic response over time, characterises "responsiveness" as the fall in blood pressure per unit concentration of drug. The significant correlations between responsiveness and pretreatment blood pressure, but not independently with age or plasma renin activity, suggest that the height of pretreatment blood pressure is a more important determinant of the magnitude of the response to antihypertensive treatment than either age or plasma renin activity (Sumner et al., 1988). This result contradicts the observation by Buhler et al. (1982) who suggested that the antihypertensive responses to calcium antagonists were increased in relation to age *per se*.

The strong correlation between the responsiveness after

the first dose and after chronic dosing might have some clinical application: for example, on the basis of the integrated data after the first dose the poor or nonresponder can be distinguished from the patient who needs a higher dose of drug.

A characteristic feature of verapamil pharmacokinetics is reduction of plasma clearance with chronic dosing. The present study has again shown that the apparent oral clearance of verapamil was reduced at steady state with a significant prolongation of elimination half life. To further investigate the underlying mechanisms liver blood flow, which is a major determinant of verapamil metabolism, has been measured in this study. There were slight and insignificant increase in apparent liver blood flow after acute and chronic verapamil administration. It is noteworthy that liver blood flow determination was made only on one occasion during the dosage interval (at the absorption phase, 1 hour after the dose) and it is not known if these changes in liver blood flow will maintain throughout the day when the plasma drug levels decline. Thus the contribution of liver blood flow to the pharmacokinetics of verapamil is not clear but it might be for short period and confined to the absorption phase. The contribution of changes in liver blood flow at the absorption phase has been studied after acute and short term dosing with nisoldipine which showed a significant correlation between changes in liver blood flow and the flow dependent part of the total AUC of nisoldipine (Van Harten et al., 1989). The pattern of increase in liver

blood flow after acute and chronic dosing of verapamil in the present study was different from that reported in young healthy volunteers (Meredith et al., 1985). Thus it would be expected that the pharmacokinetics of verapamil after acute and chronic administration must be the same if liver blood flow is the only factor responsible but since verapamil accumulates with chronic dosing in spite of the increase in liver blood flow this indicates that possibly other mechanisms such as enzyme saturation or inhibition might be involved in the mechanisms of the accumulation of verapamil at steady state. Although the ratio AUC of norverapamil/verapamil did not change between acute and chronic treatment, this provides evidence only that the N-demethylation pathway did not undergo saturation or inhibition whereas an inhibitory effect on other pathways cannot be ruled out.

In conclusion, by applying concentration-effect modelling, the antihypertensive response to verapamil has been characterised in hypertensive patients including some elderly hypertensive patients. The apparent age-related increase in responsiveness is mainly due to the higher pretreatment blood pressures of the elderly patients and there was no independent effect for age or for plasma renin activity.

CHAPTER FOUR

THE EFFECTS OF ORAL AND INTRAVENOUS DOSES OF VERAPAMIL ON EJECTION FRACTION AND P-R INTERVAL IN PATIENTS WITH STABLE ANGINA PECTORIS

INTRODUCTION:

As discussed in the previous chapter there have been reports of an age-related preferential response to calcium antagonist drugs. In addition, there are reports that age has effects on the pharmacokinetics of calcium antagonist drugs. For example Robertson et al., (1988) reported an age related reduction in the plasma clearance of nifedipine, which has been explained on the basis of an age-related decline in hepatic metabolic capacity or due to a reduction in hepatic blood flow. Regardless of the underlying mechanisms, any increase in plasma drug concentration has the potential for increasing the pharmacological responses.

If responsiveness to calcium antagonist drugs increases with age, whether as a result of increased "tissue responsiveness" or as a result of increased plasma drug levels, then not only is there potential for enhanced therapeutic effects but there is also potential for increased susceptibility to adverse effects. One such established adverse effect of verapamil is its negative effect on atrioventricular conduction (dromotropic effect) and on left ventricular contraction (inotropic effect) and accordingly there might be particular concern about the use of this drug in the treatment of middle-aged and elderly patients with angina pectoris. The principal aim of this study, therefore, was to evaluate the effects of verapamil on cardiac conduction and left ventricular function in an older patient group.

PATIENTS AND METHODS

The study was approved by the hospital Research and Ethics Committee and all patients gave written informed consent. A total of 14 patients with a clinical diagnosis of stable angina pectoris, 9 males, 5 females, body weight 74 ± 11 kg and age ranging from 42 to 76 years (60 ± 9 years) completed the study (table 4.1.). All patients were otherwise normal on clinical examination and had no clinically significant renal or hepatic insufficiency on routine blood biochemistry. Patients were allowed short acting nitrates as required but no other antianginal therapy was permitted for the duration of the study. The study design was placebo-controlled, single blind (patient blind), randomised and crossover with half the patients undergoing a placebo assessment prior to the active treatments and the other half undertaken placebo after the active treatments, following a washout period of 2 weeks. Patients attended the Clinical Pharmacology Research Unit for a total of five 8-hour study days to evaluate the effect of placebo and four active treatments. Single doses of verapamil, 5 mg intravenously and 80 mg orally, were administered on two separate occasion, one week apart, and oral treatments with 80 mg three times daily was administered for 4 weeks. At the end of the 4 week active treatment period, patients returned for a further evaluation of superimposed doses of verapamil, 5 mg intravenously and 80 mg orally, on two further separate occasions, one week apart (figure 4.1.).

TABLE 4.1.

SUMMARY OF CLINICAL DETAILS

Clinical characteristic of 14 patients with chronic stable angina pectoris.

Pat.	Sex	Age (Yrs)	Wt (kg)	History of angina (Yrs)	Additional diagnosis and drug treatment
1	M	62	76	5	Frusemide/Spironolactone
2	M	57	76	2	Old posterior MI/bendrofluazide
3	M	63	67	2	Atrial fibrillation/Warfarin
4	M	70	70	1	Aspirin
5	F	76	60	3	Mild hypertension
6	M	63	70	4	Coronary artery bypass ,1984
7	F	57	75	10	
8	M	68	75	13	Coronary artery bypass ,1979
9	F	58	75	7	Mild hypertension/bendrofluazide
10	F	64	67	1	
11	M	58	60	14	Mild hypertension/bendrofluazide
12	M	42	75	8	
13	M	57	103	3	
14	F	46	82	11	
Mean	9M	60	73.6		
Sd	5F	9	10.5		

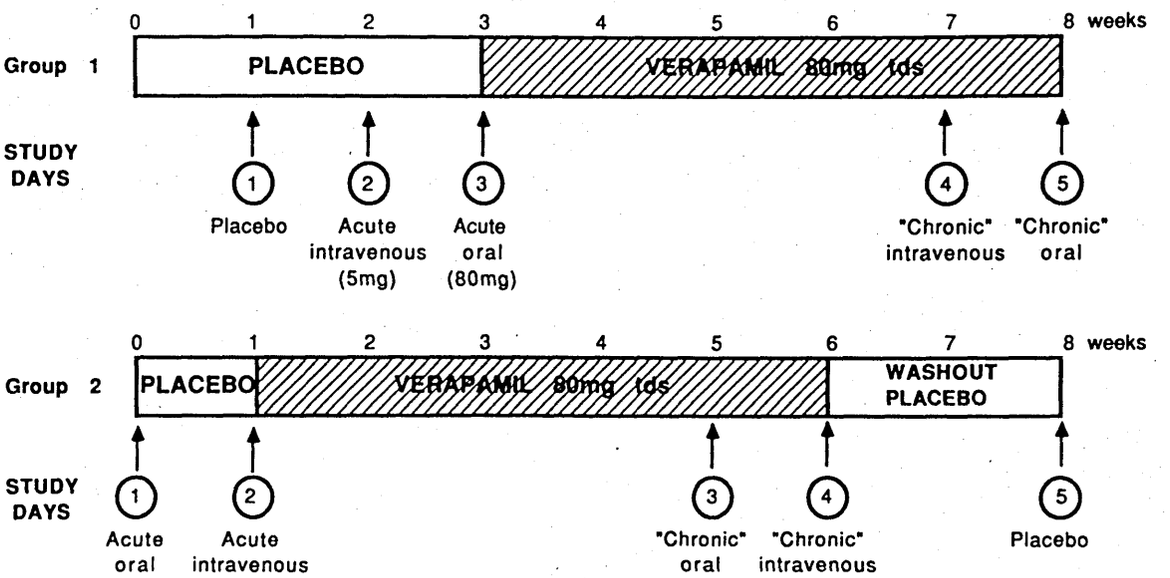


Figure 4.1. A diagram of treatments and study design

At each study day, measurement were made of the following:

a. Blood pressure and heart rate: Supine and erect blood pressure and heart rate were recorded by semiautomatic sphygmomanometer (Sentron, Bard Medical) before the dose (after 20 minutes recumbent rest) and then every hour for 8 hours.

b. Cardiac conduction: PR intervals (calculated from the departure of P wave to the beginning of Q wave) were measured from ten consecutive cardiac beats using a standard lead II electrocardiogram at a speed of 50 mm/sec. PR intervals were measured before the dose and at 1, 4, 6, 7 hours after the dose. Measurement of the PR interval at 2 and 3 hours was omitted because it coincided with the time of the evaluation of left ventricular function.

c. Left ventricular function: Left ventricular function (ejection fraction) was assessed 1.5 hours after the dose by a standard radioisotope technique, at rest and during exercise (at a fixed load of 50 watts for approximately 3 minutes). LV function was assessed on three of the five study days (placebo, after the single intravenous dose and after chronic oral dosing).

d. Apparent liver blood flow: Apparent liver blood flow was measured from the clearance of indocyanine green (ICG) following a bolus intravenous dose 0.5 mg/kg at 1 hour after the administration of placebo or active drug.

e. Renal function: Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were measured 1 hour

after the dose from the clearance of radioisotopes, ^{125}I-hippuran and ^{51}-EDTA respectively. As a result of various practical problems, full data are available for only 8 patients.

PHARMACOKINETIC ANALYSIS

Venous blood was sampled for plasma drug measurement via an indwelling intravenous cannula at times corresponding with the blood pressure recordings. Plasma was separated and stored at -20 ^{o}C until analysis. Verapamil and its principal metabolite norverapamil were assayed by an HPLC method with fluorescence detection (Cole et al., 1981).

The pharmacokinetics of verapamil and norverapamil were analysed by both model-independent and model-dependent means as described in the method section.

STATISTICAL ANALYSIS

After plotting and visually examining the data, appropriate transformation was performed if the distribution was skewed.

Repeated measures analysis of variance was used to evaluate the time and treatment effects for blood pressure, heart rate, PR interval, LV ejection fraction and apparent liver blood flow. The pharmacokinetic parameters obtained from single and multiple doses were evaluated by paired 't' test. Analysis for age related effects was made by a linear regression analysis across the age range.

Results throughout are expressed as mean $\pm$ sd.

RESULTS

1. PHARMACODYNAMICS

i) Blood pressure and heart rate

There was a tendency for all verapamil treatments to reduce blood pressure but this reduction was small and statistical significance was achieved only for supine diastolic blood pressure during chronic treatment (figure 4.2.) with an overall average reduction of 6 mmHg during the 8-hour study day.

There were similar small reductions in supine heart rate which achieved statistical significance on both study days during chronic treatment with overall reductions of 6 bpm (figure 4.2.) (69 bpm with placebo and 64 bpm with both chronic treatments) during the 8-hour study day.

ii) Cardiac conduction:

None of the active treatments was associated with any significant changes in PR interval throughout the 8-h study period (table 4.2.). From the linear regression analysis, there was no evidence of any age related effects on PR interval.

iii) LV ejection fraction

Left ventricular ejection fractions are summarised in table 4.3. There was no significant effect of either first dose (intravenous) or chronic oral dosing of verapamil on ejection fraction at rest or during exercise.

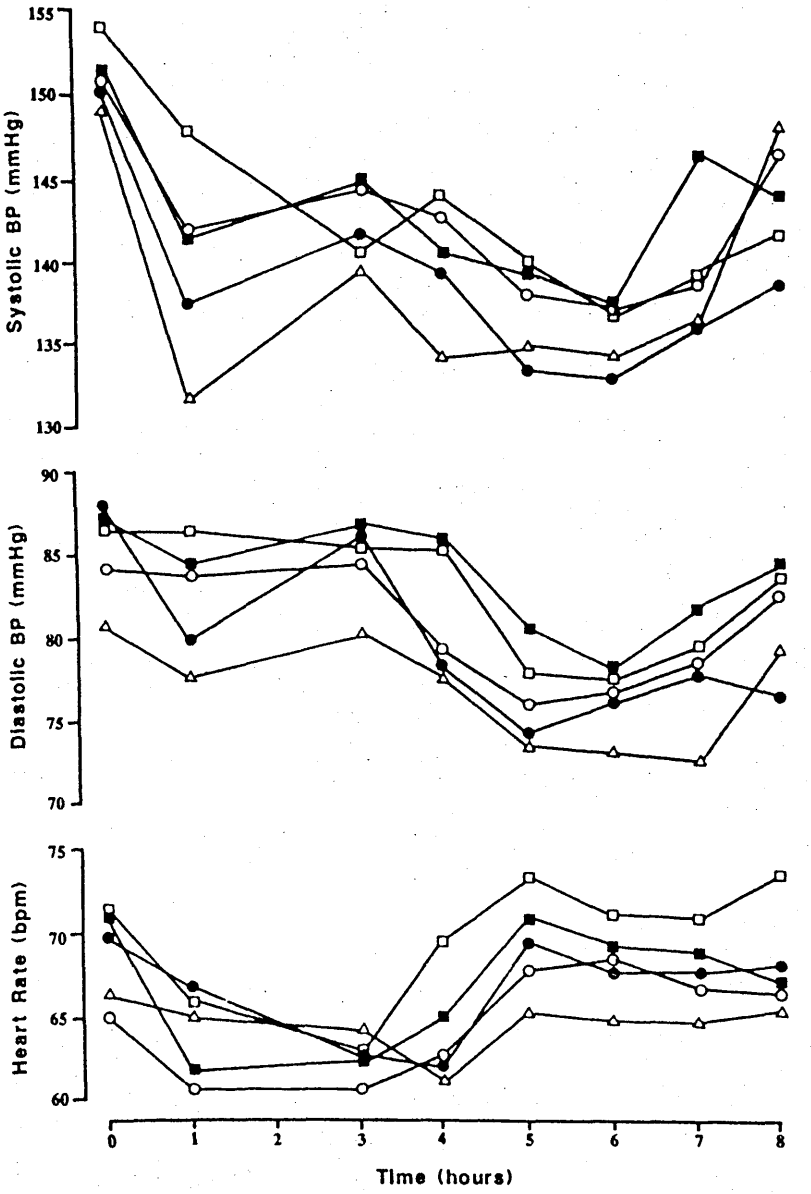


Figure 4.2. Average supine blood pressure and heart rate for all treatments . □placebo, ■acute i.v., ●acute oral, ○chronic i.v., △ chronic oral. Results of statistical analysis showed that supine diastolic blood pressure and supine heart rate achieved statistical significance during chronic treatments.

TABLE 4.2.

CARDIAC CONDUCTION
Mean PR intervals (ms)

Treatment	**Time after dosing (ms)**				
	Pretreat.	**1h**	**4h**	**6h**	**7h**
Placebo	175±22	176±17	177±22	170±20	167±18
Acute I.V. (5mg)	173±15	176±23	178±15	177±12	177±16
Acute oral (80 mg)	180±12	178±20	181±17	178±22	177±15
Chronic+I.V.	179±14	180±21	181±16	178±16	177±15
Chronic+oral	178±14	183±21	183±26	174±18	173±16

TABLE 4.3.

THE EJECTION FRACTION (%) AT REST AND EXERCISE FOLLOWING PLACEBO, SINGLE I.V. AND CHRONIC ORAL ADMINISTRATION.

	REST			DURING EXERCISE		
Pat. No.	Pla.	$I.V._{si}$	$Oral_{ss}$	Pla.	$I.V._{si}$	$Oral_{ss}$
1	28	10	36	37	24	33
2	18	16	25	27	18	nr
3	29	nr	39	34	nr	41
4	59	48	42	47	41	39
6	33	40	59	38	48	64
7	43	48	39	54	52	38
8	34	47	38	39	37	44
9	43	37	32	43	41	38
10	33	28	24	36	32	36
11	26	18	27	50	nr	38
12	27	28	37	33	35	41
13	25	24	31	32	nr	nr
14	49	50	32	75	51	61
MEAN	34.4	32.8	35.4	42	38	43
SD	11.2	14.0	9.0	12	11	10

nr: represents no results due to technical problem.
Pla: placebo; $I.V._{si}$: single intravenous dose;
$Oral_{ss}$: steady state oral dosing.

In addition, verapamil has no effect on the magnitude of change in ejection fraction during exercise. There was no independent effect of age on LV ejection fraction.

iv) Apparent liver blood flow

There was a tendency for apparent liver blood flow to increase with chronic treatment but this did not achieve statistical significance (table 4.4.). Overall there was no significant treatment effect and there was no correlation between apparent liver blood flow and age.

v) Renal function

The results of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) are presented in table 4.5. There was no significant effect attributable to any of the verapamil treatments on GFR or ERPF. Age had no independent effect on renal function.

2. PHARMACOKINETICS

i) Intravenous dosing

The plasma clearance of verapamil was significantly reduced during chronic dosing from 1.3 to 0.8 l/min and there was an associated significant prolongation of the terminal elimination half life from 3.6 to 7.9 hours ($p < 0.01$). These results are summarised in table 4.6.

The magnitude of the changes in plasma clearance (during the translation from acute to chronic dosing) tended to decline with age ($r = -0.52$, $p = 0.08$) but overall, by linear regression analysis, there was no significant age related effect on pharmacokinetics.

TABLE 4.4.

THE EFFECT OF VERAPAMIL ON LIVER BLOOD FLOW (ML/MIN)

Pat.	Placebo	$I.V._{si}$	$Oral_{si}$	$I.V._{ss}$	$Oral_{ss}$
1	1600	1800	nr	1640	1000
2	770	760	710	980	810
3	550	560	550	490	660
4	780	700	1040	750	900
5	1250	nr	1310	nr	1657
6	520	650	nr	960	1280
7	880	730	980	600	710
8	540	1120	1610	1330	2700
9	1010	1120	830	700	760
10	1300	840	990	1590	1360
11	750	560	600	600	820
12	800	1020	950	820	960
13	780	782	870	860	960
14	770	1090	900	800	960
Mean	879	902	945	932	1110
Sd	312	336	292	369	534

$I.V._{si}$: single intravenous dose; $Oral_{si}$: single oral dose
$I.V._{ss}$: chronic intravenous; $Oral_{ss}$: steady state oral dosing.
nr: represents no results due to technical problems.

TABLE 4.5.

RENAL FUNCTION

Glomerular Filtration Rate (ml/min)

Patient	Placebo	I.V.$_{si}$	Oral$_{si}$	I.V.$_{ss}$	Oral$_{ss}$
1	68	79	73	79	78
3	100	110	104	118	120
4	75	75	85	80	69
8	73	75	76	78	78
11	53	66	62	61	59
12	90	122	91	95	90
13	123	126	118	104	109
14	86	98	103	97	97
Mean	85.5	93.9	89.0	89.0	87.5
Sd	21.5	23.3	18.7	17.9	20.5

Effective Renal Plasma Flow (ml/min)

Patient	Placebo	I.V.$_{si}$	Oral$_{si}$	I.V.$_{ss}$	Oral$_{ss}$
1	294	329	339	342	269
3	220	237	359	419	455
4	370	321	418	400	379
8	271	285	304	343	340
11	184	241	201	220	207
12	413	289	415	508	428
13	393	409	444	403	326
14	414	394	435	396	420
Mean	319.9	313.1	364.4	378.8	353.0
Sd	90.1	63.7	82.6	82.4	84.9

The data are corrected for 1.73 m^2 body surface area.
I.V.$_{si}$: single intravenous dose; Oral$_{si}$: single oral dose
I.V.$_{ss}$: chronic intravenous; Oral$_{ss}$: steady state oral dosing.

There were no significant differences between different treatments on GFR or ERPF (using one way ANOVA)

ii) Oral administration

The area under the concentration time curve (AUC) was significantly increased from 429 to 868 ng/ml*h ($p < 0.01$) and this was associated with a prolongation of terminal elimination half life from 5 to 9.5 hours ($p < 0.01$).

Correspondingly the AUC of norverapamil also increased, from 252 to 875 ng/ml*h ($p < 0.01$). These results are summarised in table 4.6. Linear regression analysis for AUC during chronic dosing showed no significant correlation with patient age ($r = 0.44$) (figure 4.3.).

DISCUSSION

The putative increase in cardiovascular responsiveness to calcium antagonist drugs in the elderly might result from either altered pharmacokinetics, with increased plasma drug levels, or increased sensitivity to the pharmacological effects reflecting either a direct increase in tissue or cellular "responsiveness" or indirectly due to impaired cardiovascular reflex mechanisms (Gribbin et al., 1971).

The results of this study indicate that there was no increased susceptibility to the negative inotropic or dromotropic effects of verapamil in middle-aged and elderly patients. It might be argued that the lack of effect on cardiovascular indices could be due to low doses used in this study. However, the plasma concentrations achieved were similar to those observed in other studies which have reported significant prolongation of the PR interval (Eichelbaum et al., 1980; Dominic et al., 1981; McAllister

TABLE 4.6.

THE PHARMACOKINETICS OF VERAPAMIL

	Single dose	Chronic dosing	Difference
Intravenous dosing			
Clearance (Cl) (l/min)	1.3 ± 0.3	0.8 ± 0.5	-0.5 ± 0.5 b
Volume of distribution (Vdss) (l)	346.0 ± 98	410.0 ± 240	58.0 ± 23 ns
Elimination half-life ($t_{1/2}$) (h)	3.6 ± 1	7.9 ± 3	4.4 ± 3.2 d
Oral dosing			
AUC-verapamil (ng/ml*h)	429.0 ± 219	868.0 ± 422	438.0 ± 287 d
Clearance/F (l/min)	4.1 ± 2.5	1.9 ± 0.9	-2.1 ± 1.7 c
Elimination half-life ($t_{1/2}$)	5.0 ± 1.6	9.5 ± 5.4	4.5 ± 5.5 a
C_{max} (ng/ml)	74.0 ± 29	165.0 ± 68	90.0 ± 52 d
T_{max} (h)	1.7 ± 0.8	1.4 ± 0.5	-0.3 ± 0.9 ns
AUC-norverapamil (ng/ml*h)	252.0 ± 97	875.0 ± 353	622.0 ± 285 d

a: $p < 0.05$ b: $p < 0.01$ c: $p < 0.001$ d: $p < 0.0001$

ns: not significant.

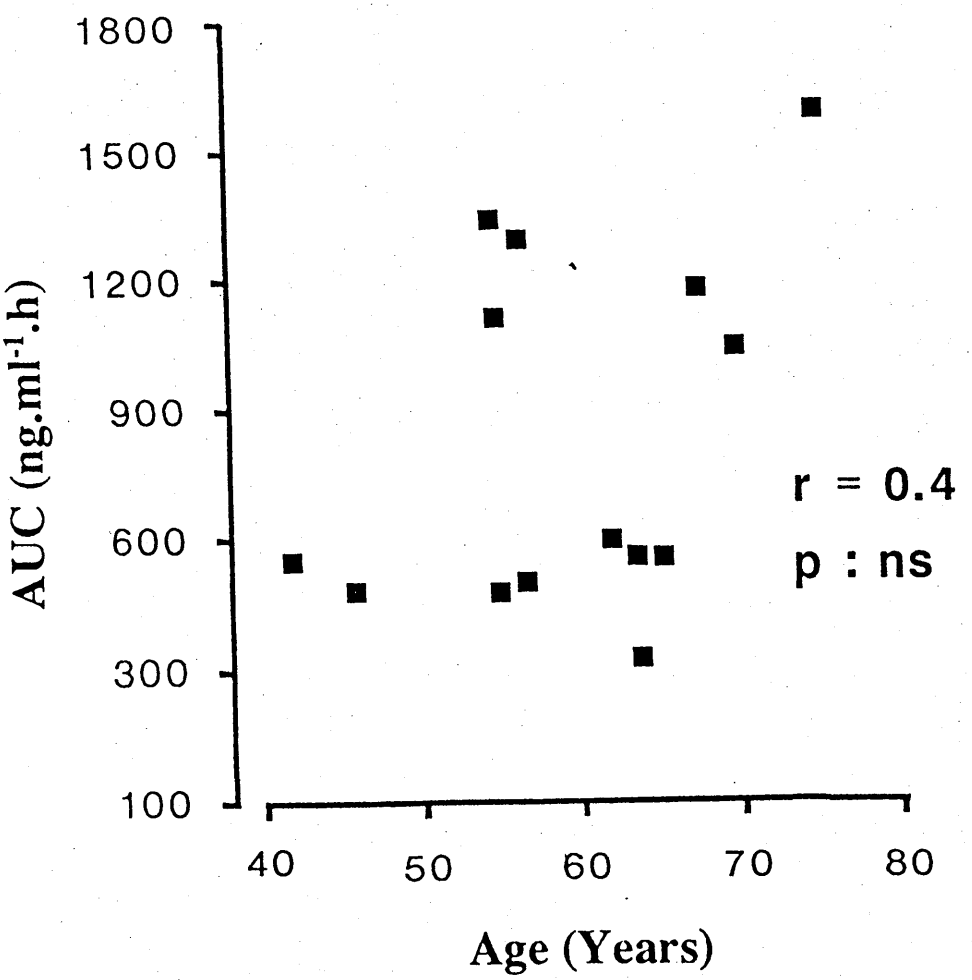


Figure 4.3. Relationship between age and the area under the concentration time curve (AUC_{0-8h}) during chronic dosing.

Y = 20.67*X -370

95% C.I. of slope = -5.8, 47.1

et al., 1982). In these studies, significant prolongations in the PR interval were obtained in healthy young volunteers within the concentration range 30-50 and 50-150 ng/ml following intravenous and oral verapamil respectively. Thus with clinically recommended doses, the mean C_{max} values (the peak plasma concentration) of 74 and 165 ng/ml in this study might have been expected to produce significant prolongation of PR interval particularly in those older patients if there was an increased sensitivity to the drug's actions.

Prolongation of PR interval may constitute a "rough" guide in the clinical use of verapamil and indicate that potentially therapeutic drug levels have been achieved; the presence of second or third degree AV block suggests drug toxicity and implies excessive plasma drug concentrations (Dominic et al., 1981). However, this would appear to have limited application in the elderly who, in the absence of a measurable effect on PR interval, were still able to demonstrate small but significant haemodynamic effects with reductions in supine blood pressure and in supine heart rate during chronic verapamil dosing.

The mechanism underlying the accumulation of verapamil with continued administration also has been investigated in this study. After oral administration, a high concentration of verapamil is delivered to the liver via the portal vein and there is the possibility of saturating the capacity of the hepatic enzymes (Wagner et al., 1984). After intravenous administration relatively low concentrations are delivered to the liver, mainly through

the hepatic artery with each cardiac cycle. In this study oral dosing with verapamil was continued for 4 weeks and a supervised intravenous dose was then administered. The plasma clearance of verapamil after this intravenous dose was reduced compared to the clearance after the first intravenous dose. It is unlikely, even with steady state verapamil, that the pre-dose concentrations would be sufficiently high to have already saturated the enzymes. The more likely explanation is that verapamil inhibits the enzyme(s) responsible for its metabolism and that this will only be reversed when drug is cleared from the metabolising sites following cessation of treatment.

Although, these patients with ischemic heart disease generally had low LV ejection fractions, there was no, significant effect attributable to verapamil. Moreover verapamil prevented any reduction in ejection fraction during exercise. This result is in agreement with the results of other studies in which oral daily doses of 320 and 480 mg verapamil (Tan et al., 1982; Josephson et al., 1981) or an intravenous dose (0.06 mg/kg) (Klein et al., 1983) were used . These studies have not only shown that verapamil prevented the exercise-induced reduction in ejection fraction but it also reduced the number of exercise induced abnormalities in regional wall motion.

From animal studies it appears that the concentration range for the negative inotropic effect is approximately 3-folds greater than the range for negative chronotropic effect (Hof et al., 1983). Such a range of concentration is

unlikely to be achieved in routine clinical practice.

There is evidence from human studies that the pharmacokinetics of verapamil (Abernethy et al., 1986) and of dihydropyridine calcium antagonists (Robertson et al., 1988; Donnelly et al., 1988; Abernethy et al., 1988) change with increasing age leading to increased peak plasma levels and increased AUC. It has been assumed that this reflects an age-related decline in hepatic metabolic capacity and, although there is no direct evidence in man, there is experimental evidence in the rat that hepatic metabolic capacity declines with age (Kato & Takanaka, 1968). In this present study, although there was a tendency for the plasma clearance of verapamil to decline in relation to increasing age, none of the calculated pharmacokinetic parameters showed any significant age-related differences. This study, however was designed to examine the population likely to have ischemic heart disease and to require treatment with verapamil. Since such patients would generally fall into the 40-75 years age group, as in this study, the lack of significant age-related differences may simply reflect the age range studied. A very broad age range (23-102 years) was studied by Abernethy et al., 1986 to demonstrate significant age-related differences in the disposition of verapamil but other studies, either with fewer subjects (Norris et al., 1987) or with a narrower age range (19-79 years) (chapter 5) have failed to confirm a significant age-related decline in the clearance of verapamil. Thus, while the age range studied may explain the failure to detect a significant

age-related effect on the disposition of verapamil, there appears to be no need for major adjustments in the patient population likely to receive verapamil in routine clinical practice. Clearly the full dose-concentration range was not explored in this study and no attempt was made to define an anti-anginal therapeutic range. The principal findings of this study are that middle-aged and elderly patients with ischemic heart disease do not show exaggerated response to the cardiac effects of verapamil.

CHAPTER FIVE

THE INFLUENCE OF AGE ON THE PHARMACOKINETICS OF VERAPAMIL

INTRODUCTION

The ageing process is associated with a range of physiological changes which might be expected to affect drug disposition and pharmacokinetics.

The systemic clearance of verapamil, which undergoes high hepatic extraction, depends on two major factors: liver blood flow and the integrity of the hepatic metabolising enzymes. While there is a good evidence that liver blood flow declines with age (Bender et al., 1965; Wynne et al., 1988), there is only indirect evidence from studies in rats that the activity of the metabolising enzymes is reduced with age (Kato & Takanaka, 1968). Thus there is a considerable background evidence to suggest that increasing age might affect the disposition of verapamil. The pharmacokinetics of verapamil have been clearly defined in healthy volunteers (Freedman et al., 1981; Shand et al., 1981; Meredith et al., 1985) but there is only limited information about the steady state disposition of verapamil in the elderly.

SUBJECTS AND METHODS

The data for this analysis were collected from a series of studies conducted in the Clinical Pharmacology Research Unit (C.P.R.U) of Stobhill Hospital. All studies followed a similar protocol and, in particular, the sampling time schedules and drug assay methods were identical. Seventy four subjects were studied including patients with mild to moderate essential hypertension (n=51) or stable angina

pectoris (n=13) and healthy normotensive volunteers (n=10). No subject had clinically significant renal or hepatic insufficiency on routine blood biochemistry. The study population was arbitrarily sub-divided into 3 age groups and their demographic details are given in table 5.1. Group 1 ranged in age from 19 to 44 years (n=26) and was designated the "young" age group; Group 2 ranged from 45 to 64 years (n=27) and was designated "middle-aged" group; and Group 3 ranged from 65 to 79 years (n=21) and was designated the "elderly" group. All individuals were established on a twice daily regimen with verapamil, 240 or 360 mg total daily dose, for at least 1 week prior to study. On the study day subjects reported fasting to the C.P.R.U. to receive a supervised dose of 120 or 180 mg verapamil orally and thereafter to have withdrawal of venous blood samples. Blood samples were collected onto ice and immediately centrifuged to separate off the plasma which was then stored at -20 C for subsequent analysis.

Plasma drug analysis

Concentrations of verapamil and norverapamil were analysed by HPLC with fluorescence detection using the method of Cole et al. (1981)

Pharmacokinetic analysis

The pharmacokinetic parameters of verapamil such as the AUC, clearance/F, elimination half life, t_{max} and C_{max} were calculated by standard techniques described in the methods chapter.

Statistical analysis

Normality of distribution was tested by the X^2 test. Appropriate transformations of data were required for age, $t_{1/2}$ and t_{max}. Age related effects were evaluated by applying linear regression analysis across the age range and by one way analysis of variance following the arbitrary division into three groups.

RESULTS

The derived pharmacokinetic characteristics are summarised in table 5.1.

There was a significant increase in maximum plasma concentration (C_{max}) with increasing age (r=0.24, t=2.16, p=0.03) (figure 5.1.). The C_{max} was 400 $\pm$ 197 ng/ml in the elderly group compared to 270 $\pm$ 86 and 348 $\pm$ 189 ng/ml in the young and middle aged group respectively. The time to attain the maximal concentration (t_{max}) was not significantly changed with increasing age (figure 5.2a.) and although there was a trend for the ratio $AUC_{norverapamil}/AUC_{verapamil}$ to increase with age this was not statistically significant (figure 5.2b.)

Although terminal elimination half life was apparently longer in the elderly group, 9.8 $\pm$ 6 hours, compared to 8.0 $\pm$ 3.6 in the young group, there was no significant age related increase in terminal elimination half life across the whole age range (figure 5.3a.) (r=0.11, t=0.95, p=0.34). There was no significant correlation between age and clearance/F (figure 5.3b) although clearance/F tended to decline with increasing age (r=-0.2, t=1.73, p=0.08) and the lowest value, 91 $\pm$ 46 l/h, was obtained in the elderly group.

TABLE 5.1.

SUMMARY OF THE DEMOGRAPHIC DATA AND THE DERIVED PHARMACOKINETIC PARAMETERS (MEAN + SD)

Group	**1**	**2**	**3**	**Total**
Age range	19-44	45-64	65-79	19-79
Mean age	31	58	73	53
Number of subjects	26	27	21	74
Weight (kg)	70 ± 7	70 ± 1	66 ± 11	69 ± 10
C_{max} (ng/ml)	270 ± 86	348 ± 189	400 ± 197	335 ± 169
t_{max} (h)	1.2 ± 0.3	1.6 ± 0.9	1.6 ± 1	1.4 ± 0.8
$t_{1/2}$ (h)	8.0 ± 3.6	9.3 ± 4	9.8 ± 6.1	9.0 ± 4.6
CL/F (l/h)	109.7 ± 25	117.5 ± 52	90.5 ± 46	106 ± 43
(l/h/kg)	1.57 ± 0.4	1.64 ± 0.6	1.34 ± 0.6	1.53 ± 0.54
AUC_{norver}/AUC_{ver}	0.96 ± 0.2	1.01 ± 0.2	1.03 ± 0.2	0.99 ± 0.17

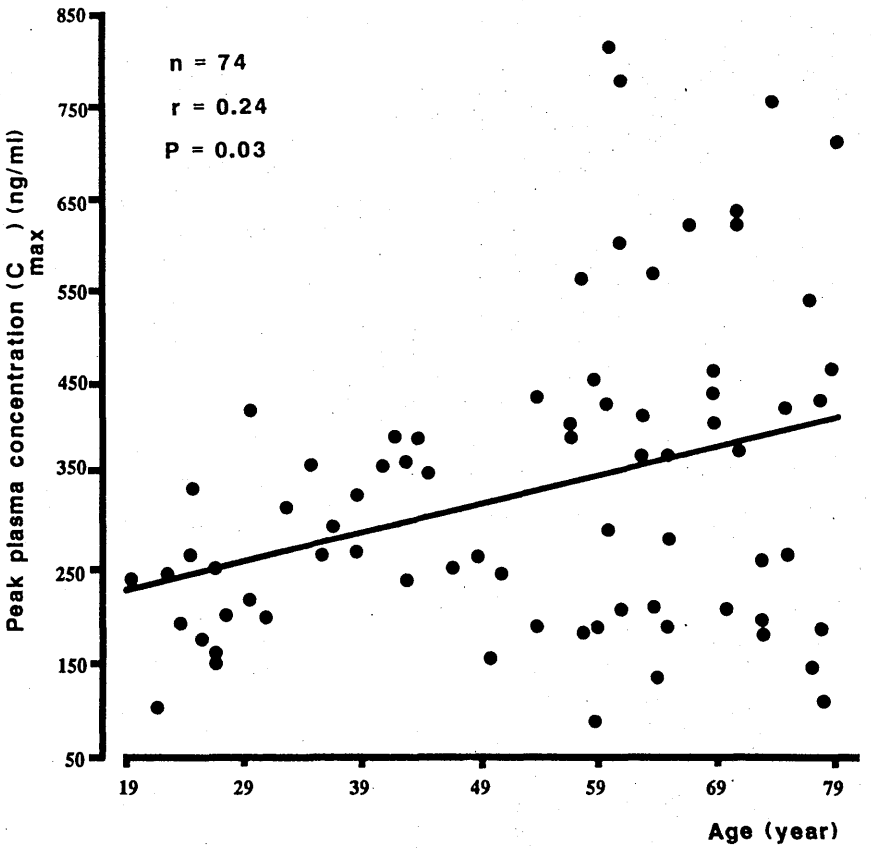


Figure 5.1. Relationship between age and the peak plasma concentration (C_{max}) of verapamil at steady state.

slope=8.6×10^{-7};
95% confidence interval of slope=8.2×10^{-8}, 1.6×10^{-6};
Power of correlation coefficient (r) = 69%

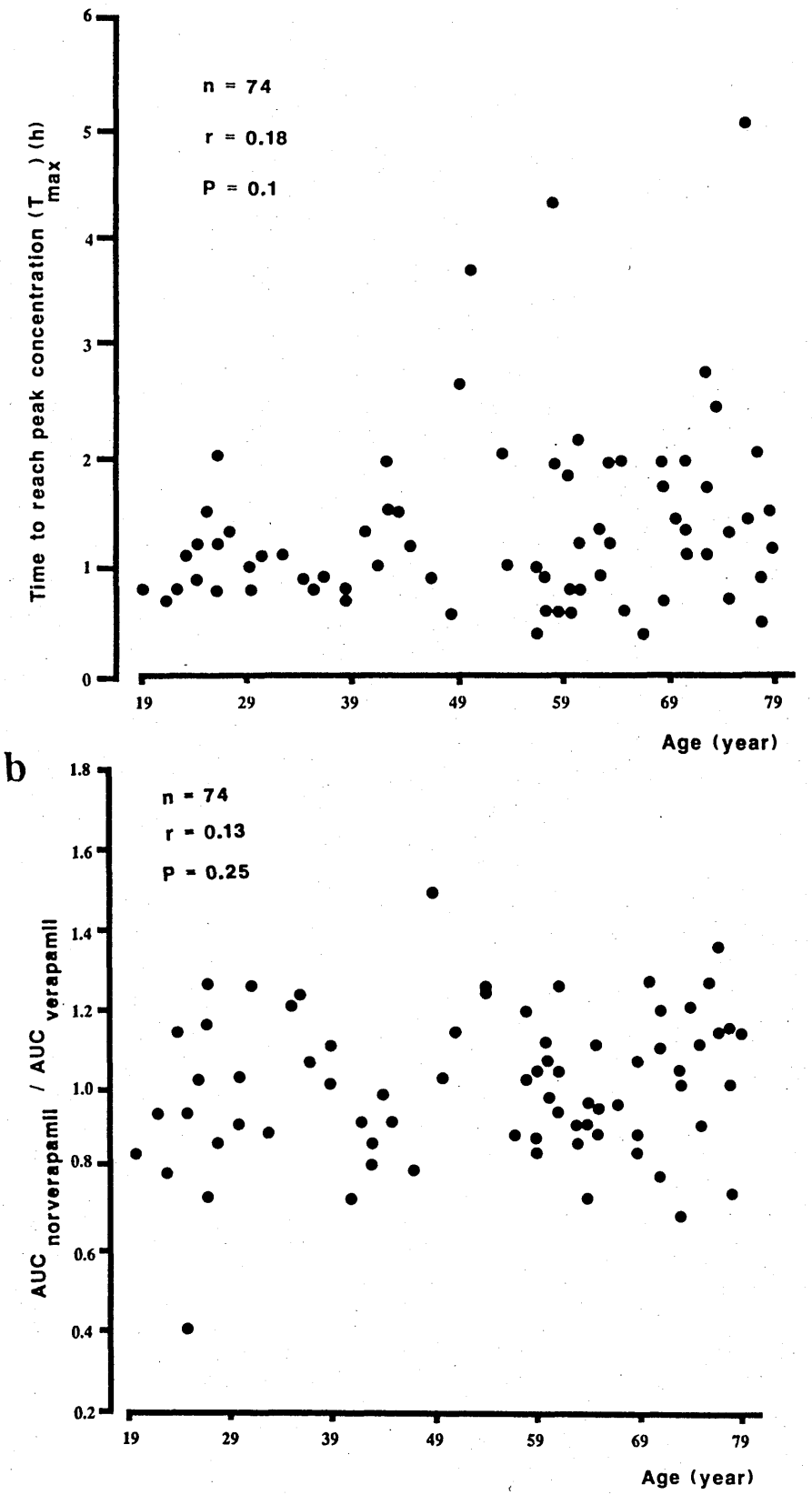


Figure 5.2. Relationship between age and time to reach peak concentration (t_{max}) (a) and age and the ratio of $AUC_{norver.}/AUC_{ver.}$ at steady state (b).

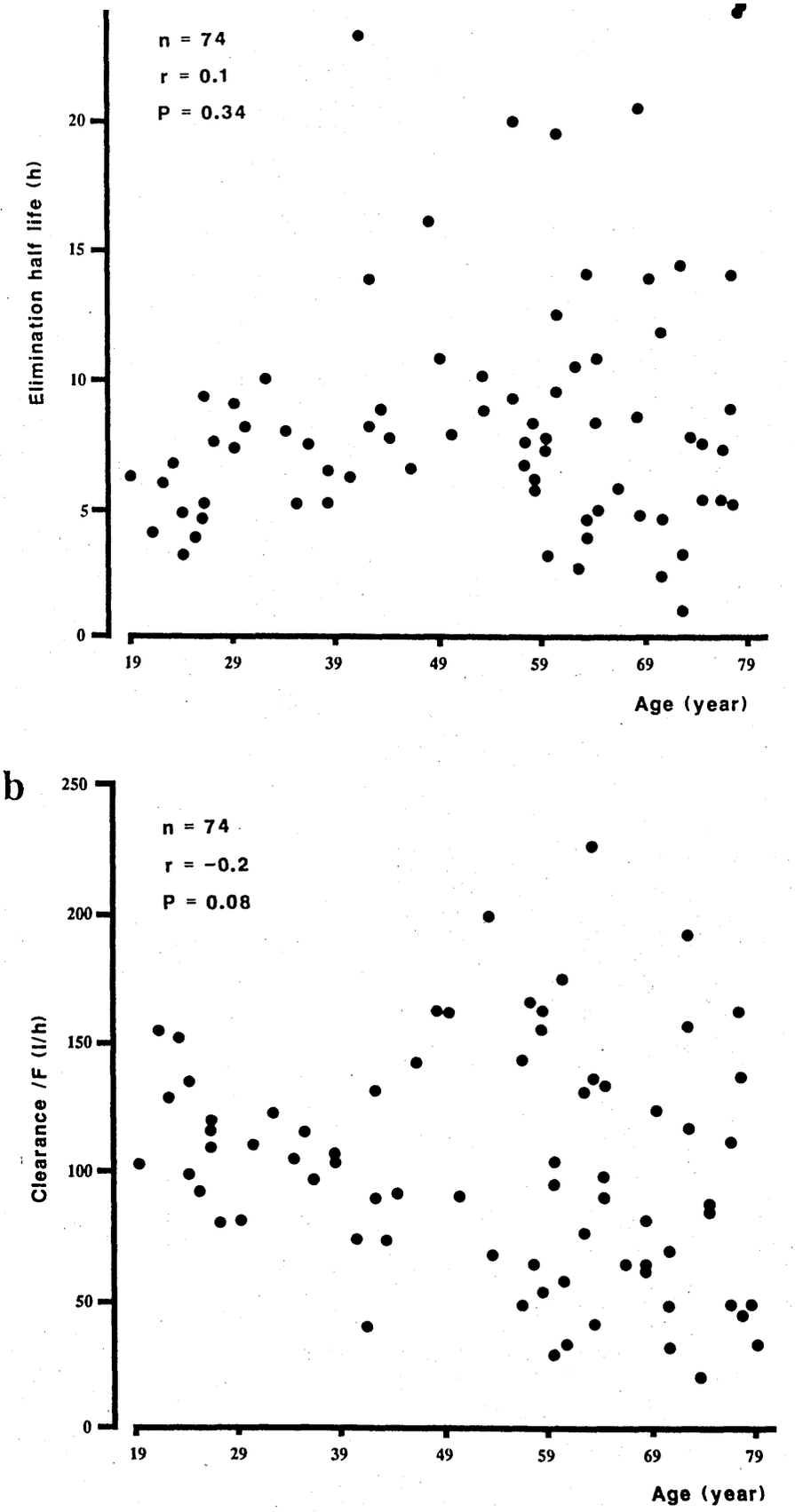


Figure 5.3. Relationship between age and the elimination half life (a); age and clearance/F (b) of verapamil at steady state.

DISCUSSION

Overall, the results of this analysis suggest that there are small age-related alterations in the disposition of verapamil. A statistically significant change was found for peak plasma concentrations and there were non-significant trends for age related changes in apparent oral clearance and elimination half life.

These findings are consistent with the findings of other studies which have investigated the effect of increasing age on the pharmacokinetics of calcium antagonist drugs. For example, with dihydropyridine calcium antagonists, Robertson et al. (1988) have reported a reduction with age in the plasma clearance of nifedipine, Donnelly et al., (1988) have reported an age-related increase in C_{max} for nifedipine and Abernethy et al. (1988) and Elliott et al. (1988) have reported age-related changes in the clearance and terminal elimination half life of amlodipine. Abernethy et al. (1986) have also described a reduction in the clearance of intravenous verapamil in elderly and very elderly hypertensive patients when compared to young patients. A similar nonsignificant trend for an age-related change in apparent oral clearance has been also described (Norris et al., 1987). While the most probable explanation for these pharmacokinetic differences lies with altered hepatic function, it remains unclear whether this reflects age-related changes in liver blood flow or in intrinsic metabolic capacity. Additionally, in the present study, it is not possible to exclude changes in volume of

distribution.

An obvious problem with this type of evaluation is the difficulty in quantifying the contribution of age independently from a variety of other genetic, pathological and environmental factors. For example, in a large study reported by Vestal, 1975, the clearance of antipyrine which is entirely metabolised by the liver, was found to be significantly reduced with age. However, age per se accounted for only 3% of the variability which was otherwise attributable to smoking and other incidental factors (Vestal 1975). In this present study there was a nonsignificant reduction in clearance/F of verapamil and associated with this there were significantly higher peak plasma levels in the elderly. This may be of clinical relevance insofar as some of the pharmacological effects of verapamil are directly related to plasma concentrations and there might be increased risk of side effects.

Increasing age is also associated with increasing inter-subject variability, as can readily be seen in all the figures. Such heterogeneity in the data may itself confound interpretation as illustrated by the absence of an overall correlation between Cl/F and age, whereas there was a significant correlation between age and clearance/F in the young subjects (age range 19-45 years) ($r=-0.47$, $p=0.02$, $n=26$) when the correlation was separately examined for each age group, but no significant correlation for middle-aged or elderly subjects. This age-related increase in variability will have two important consequences. Firstly, statistical

confirmation of the age-related differences will be compromised unless relatively large numbers are studied. Secondly, it makes it inappropriate to make global dosage recommendations for the older age groups.

In summary, these results suggest that increasing age is associated with alterations in the pharmacokinetics of verapamil. This is consistent with the evidence from other studies and is consistent with an age-related decline in drug clearance. However, although this small but statistically significant age-related difference has been confirmed it seems unlikely that it will have major clinical significance.

CHAPTER SIX

A STUDY OF THE PHARMACOKINETICS OF D-VERAPAMIL AND ITS EFFECTS ON PR INTERVAL, BLOOD PRESSURE AND HEART RATE

INTRODUCTION

The calcium antagonist drugs verapamil, diltiazem, nicardipine, nifedipine, niludipine, nimodipine and other unrelated compounds, such as amiodarone and quinidine, have been shown to enhance the cytotoxicity of natural product anticancer drugs such as vinca alkaloids and anthracyclines in Multiple Drug Resistance (MDR) cancer cells (Tsuruo et al., 1983b; Beck et al., 1985; Harker et al., 1986)

The mechanism underlying the development of resistance is not well defined although several investigators attribute it to decreased retention of the cytotoxic drug inside the cell as a result of an "active efflux pump" (Skovsgaard, 1978; Inaba et al., 1981). Since the hallmark of MDR cells is an increase in the amount of a high molecular-weight surface glycoprotein, p-glycoprotein (P-180), (Kartner et al., 1985) it is suggested that this leads to alterations in the cell membrane and increased transfer of drugs across the membrane (Riordan & Ling 1985). The mechanism by which adjuvant drugs, such as verapamil, reduce the development of resistance is also unknown. However, with the conventional (i.e. racemic) form of verapamil the drug concentration required to overcome MDR cells *in vitro* is relatively high (in the range 2-6 μM: 1000 - 3000 ng/ml, approximately) (Merry et al., 1989). Such concentrations are difficult to achieve *in vivo* in man without serious cardiovascular side effects, particularly depression of atrioventricular conduction (i.e. negative dromotropism).

Racemic verapamil, however, is a mixture of d- and l-isomers and previous studies have shown that d-verapamil is 8-10 times less potent than l-verapamil in terms of its effect on cardiac conduction and prolongation of PR interval (Echizen et al., 1985, Echizen et al., 1988). D-verapamil has been shown to have activity similar to the racemic formulation of verapamil as a modulator in the treatment of cancer (Plumb et al., 1989). Therefore, it may be possible to achieve the required high plasma concentrations with less cardiovascular side effects, by administering the single d-isomer. The principal aim of the study, therefore, was to investigate the pharmacokinetics and pharmacodynamics of relatively high doses of d-verapamil in healthy volunteers.

SUBJECTS AND METHODS

Eight healthy normotensive male volunteers, aged 27 ± 7 years, range 19-40 years; body weight 74.5 ± 6.5 kg. (table 6.1.) gave written informed consent to participate in the study which had received prior approval from the Hospital Research and Ethical Committee. All subjects were deemed normal on the basis of routine clinical and laboratory examinations with pre-study electrocardiogram (ECG) examinations which confirmed normal sinus rhythm and a PR interval not greater than 180 ms.

The 8 subjects were sub-divided into 2 groups of 4. Subjects in group 1 attended the Clinical Pharmacology Research Unit (CPRU) at weekly intervals for a series of four 24-h study days to receive single doses of placebo, 240

mg racemic verapamil, 250 mg d-verapamil and 500 mg d-verapamil. The study design was double blind but the randomisation was incomplete such that the 500 mg dose of d-verapamil was, for safety reason, always administered as the fourth treatment, following screening (blinded) of the blood pressure, heart rate and PR interval data from the previous study days. Subjects in group 2 followed the same overall study design with placebo and 240 mg racemic verapamil but with doses of 500 mg and 1000 mg d-verapamil.

On each study day, subjects reported to the CPRU having abstained from food, alcohol, nicotine and caffeine since 10 pm the previous evening. An intravenous cannula was introduced for blood sampling and after 20 minutes recumbency baseline measurements of blood pressure and heart rate were recorded by semiautomatic sphygmomanometer and a standard lead II ECG was recorded at a speed of 50 mm/sec for the measurement of PR interval. Following oral dosing with verapamil or placebo, along with 150 ml of water, measurements of blood pressure, heart rate and PR interval were repeated at times 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours.

Plasma drug analysis

For both racemic and d-verapamil, concentrations of verapamil and norverapamil were analysed by HPLC with fluorescence detection after the method of Cole et al (1981).

TABLE 6.1.

CLINICAL CHARACTERISTIC OF VOLUNTEERS

Subjects	wt (kg)	age (years)	PR (ms)	BP. (mmHg)	HR (bpm)
Group 1					
1	72	22	168	125/62	63
2	68	19	124	159/91	79
3	68	40	178	119/74	76
4	78	35	150	126/91	91
Group 2					
1	67	25	128	128/69	65
2	80	26	175	136/74	62
3	83	27	143	117/59	61
4	80	22	167	137/85	67

Pharmacokinetic analysis

The concentration time profiles of racemic verapamil and d-verapamil and their respective metabolites were best fitted to a two compartment model with a third compartment for metabolite.

The area under the concentration time curve (AUC), clearance/F and terminal elimination half life were obtained by standard techniques, as described in the methods chapter.

Prediction of steady state concentrations

Using the parameters obtained from the single dose fitting in each individual subjects, a model was derived to predict steady state concentrations. The model for steady state comprised two compartments for drug with first order input from the gut and one compartment for metabolite. Predictions were based upon dosage intervals of two, three and four times daily administration. Since it is known that the clearance of racemic verapamil decreases during continued administration, the predictive model has been modified to incorporate a "correction" factor of 2.4, as suggested by Wagner et al. (1984). This model takes account not only of the prolongation of elimination half life of verapamil but also of its major metabolite, norverapamil which also has been shown to accumulate with multiple doses.

Statistical analysis

Analysis of variance (ANOVA) has been used to evaluate treatment and time effects for repeated measurements of

blood pressure, heart rate and PR intervals. This test has been applied on the separate groups (n = 4) and also on the two groups combined (n = 8) for the common treatments - placebo, 240 mg racemic verapamil and 500 mg d-verapamil. A P value of less than 0.05 was considered significant.

RESULTS

1. PHARMACOKINETICS

Peak plasma concentrations (C_{max}) and times to attain the peak (t_{max}) are presented for all doses in table 6.2.

There were dose-related increases in C_{max}, 308 and 640 ng/ml in group 1 and 1143 and 2103 ng/ml in group 2 and no significant changes in t_{max} which occurred between 1-2 hours after the dose.

The fitted parameters for verapamil and norverapamil are presented in tables 6.3. and 6.4. The AUC for d-verapamil increased as the dose increased (table 6.5.) but at the highest dose of 1000 mg there was a tendency to nonlinearity with a mean value of 11818 ng/ml*h which is about 22% more than would have been projected from the 4835 ng/ml*h obtained with 500 mg.

Apparent oral clearance (clearance/F) is presented in table 6.6. and, as indicated by the AUC itself, the lowest clearance value of 1.6 $\pm$ 0.5 l/min occurred after a 1000 mg dose. Elimination half life did not differ between doses and ranged between 4 and 6 hours (table 6.7.).

TABLE 6.2.

PEAK PLASMA CONCENTRATION (C_{max}) AND TIME TO REACH PEAK (T_{max}) OF VERAPAMIL

GROUP 1

	240 mg Racemic		250 mg -V d-isomer		500 mg -V d-isomer	
Subject	**C_{max}**	**t_{max}**	**C_{max}**	**t_{max}**	**C_{max}**	**t_{max}**
1	321	1.5	233	1.0	621	1.5
2	236	1.0	450	1.5	526	3.0
3	432	1.0	309	2.0	807	1.0
4	294	1.0	241	1.0	607	1.5
Median	307	1.0	275	1.3	614	1.5
Mean	321	1.1	308	1.4	640	1.7
Sd	82	0.25	100	0.5	119	0.9

GROUP 2

	240 mg Racemic		500 mg -V d-isomer		1000 mg -V d-isomer	
Subject	**C_{max}**	**t_{max}**	**C_{max}**	**t_{max}**	**C_{max}**	**t_{max}**
1	261	1.5	876	1.5	2029	2.0
2	353	1.0	621	1.5	1586	1.5
3	318	1.0	1042	1.5	1723	1.5
4	636	1.0	2033	1.0	3074	1.0
Median	336	1.0	959	1.5	1876	1.5
Mean	392	1.1	1143	1.4	2103	1.5
Sd	167	0.2	618	0.2	673	0.4

TABLE 6.3.

PHARMACOKINETICS OF VERAPAMIL

GROUP 1

240 mg Racemic verapamil

Sub.	A	Alpha	B	Beta	Ka	Tlag	Km0	R^2
1	301	0.8	209	0.13	4.5	0.5	1.3	0.980
2	151	1.1	99	0.13	10.3	0.5	0.6	0.946
3	742	2.8	266	0.21	7.4	0.5	0.7	0.957
4	168	0.7	161	0.18	11.3	0.6	0.7	0.968
Median	234	0.9	185	0.16	8.8	0.5	0.7	
Mean	340	1.3	184	0.16	8.4	0.5	0.8	
Sd	239	0.9	62	0.03	2.7	0.02	0.3	

250 mg d-verapamil

1	239	0.4	81	0.09	4.0	0.5	4.8	0.969
2	254	0.2	287	0.15	2.6	0.4	1.4	0.925
3	126	0.7	815	0.12	1.5	0.8	1.7	0.982
4	127	1.2	144	0.16	10.9	0.5	2.2	0.886
Median	183	0.6	216	0.14	3.3	0.5	1.9	
Mean	187	0.6	332	0.13	4.7	0.5	2.5	
Sd	60	0.4	289	0.03	3.7	0.2	1.4	

500 mg d-verapamil

1	368	0.3	318	0.25	19.8	1.0	1.5	0.949
2	499	0.2	449	0.16	0.9	0.8	1.3	0.955
3	646	0.5	311	0.15	58.0	0.5	1.0	0.977
4	394	0.6	433	0.15	3.1	0.5	0.9	0.959
Median	446	0.4	376	0.16	11.4	0.7	1.1	
Mean	477	0.4	378	0.18	20.0	0.7	1.2	
Sd	109	0.2	64	0.04	23.0	0.2	0.3	

A & B are coefficients; Alpha, Km0 & Beta are elimination rate constants; Ka is absorption rate constant; Tlag is the time befo: the drug is detected in plasma; R^2 is coefficient of determinati

TABLE 6.4.

PHARMACOKINETICS OF VERAPAMIL

GROUP 2

240 mg Racemic verapamil

Sub.	A	Alpha	B	Beta	Ka	Tlag	Km0	R^2
1	286	0.4	571	0.22	0.6	0.5	0.9	0.946
2	305	1.0	139	0.14	10.8	0.6	0.6	0.935
3	195	0.8	201	0.15	17.0	0.5	1.4	0.966
4	2441	1.8	292	0.16	3.0	0.3	0.6	0.957
Median	296	1.0	301	0.16	6.9	0.5	0.8	
Mean	807	1.0	301	0.17	7.8	0.5	0.9	
Sd	944	0.5	165	0.03	6.5	0.1	0.3	

500 mg d-verapamil

1	1981	5.9	1043	0.21	3.9	0.2	2.3	0.957
2	1144	0.7	387	0.16	1.8	0.3	1.0	0.966
3	1272	0.6	505	0.16	2.3	0.5	1.8	0.954
4	3138	1.0	922	0.17	3.4	0.4	0.7	0.965
Median	1627	0.9	714	0.17	2.8	0.3	1.4	
Mean	1884	2.0	714	0.17	2.8	0.3	1.5	
Sd	711	2.2	275	0.02	0.8	0.1	0.6	

1000 mg d-verapamil

1	1734	0.2	1145	0.19	2.2	0.5	0.8	0.954
2	1802	0.5	599	0.13	2.2	0.4	1.9	0.956
3	3288	0.6	647	0.09	1.5	0.4	1.0	0.954
4	1647	0.5	2896	0.14	3.3	0.4	0.7	0.975
Median	1768	0.5	896	0.14	2.2	0.4	0.9	
Mean	2118	0.4	1322	0.14	2.3	0.4	1.1	
Sd	678	0.1	934	0.03	0.6	0.04	0.4	

A & B are coefficients; Alpha, Km0 & Beta are elimination rate constants; Ka is absorption rate constant; Tlag is the time before the drug is detected in plasma; R^2 is coefficient of determination.

TABLE 6.5.

AREA UNDER THE CONCENTRATION TIME CURVE ($AUC_{0-\infty}$ NG/ML*H) (CALCULATED BY TRAPEZOIDAL RULE)

GROUP 1

	240 mg Racemic		250 mg -V d-isomer		500 mg -V d-isomer	
Subject	**Ver**	**Nor**	**Ver**	**Nor**	**Ver**	**Nor**
1	1819	816	1376	808	2501	2160
2	1810	1098	2983	1853	3969	3942
3	1304	1021	1563	1196	2865	2902
4	1003	1138	907	957	3115	3689
Median	1557	1059	1470	1077	2990	3295
Mean	1484	1018	1707	1203	3113	3173
Sd	347	124	774	400	540	700

GROUP 2

	240 mg Racemic		500 mg d-isomer		1000 mg d-isomer	
Subject	**Ver**	**Nor**	**Ver**	**Nor**	**Ver**	**Nor**
1	2368	1940	4496	3722	13480	9368
2	1208	1185	3089	2459	7698	5953
3	1368	1174	4002	3757	8975	8032
4	2550	2083	7754	5780	17119	11154
Median	1868	1562	4249	3739	11227	8700
Mean	1873	1595	4835	3929	11818	8627
Sd	592	419	1759	1189	3739	1900

TABLE 6.6.

CLEARANCE/F OF VERAPAMIL (L/MIN)
(CALCULATED BY DIVIDING DOSE BY $AUC_{0-\infty}$)

GROUP 1

Subject	240 mg Racemic	250 mg -V d-isomer	500 mg -V d-isomer
1	2.2	3.0	3.3
2	2.2	1.4	2.1
3	3.1	2.7	2.9
4	4.0	4.6	2.7
Median	2.7	2.9	2.8
Mean	2.9	2.9	2.7
Sd	0.7	1.1	0.4

GROUP 2

Subject	240 mg Racemic	500 mg -V d-isomer	1000 mg -V d-isomer
1	1.7	1.8	1.2
2	3.3	2.7	2.2
3	2.9	2.1	1.9
4	1.6	1.1	1.0
Median	2.3	1.9	1.6
Mean	2.4	1.9	1.6
Sd	0.8	0.6	0.5

TABLE 6.7.

TERMINAL ELIMINATION HALF LIFE OF VERAPAMIL AND NORVERAPAMIL $T_{1/2}$ (HOURS)

GROUP 1

	240 mg Racemic		250 mg -V d-isomer		500 mg -V d-isomer	
Subject	**Ver**	**Nor**	**Ver**	**Nor**	**Ver**	**Nor**
1	5.4	0.5	7.7	0.1	2.8	0.5
2	5.3	1.2	4.6	0.5	4.3	0.5
3	3.3	1.0	5.8	0.4	4.6	0.7
4	3.9	1.0	4.3	0.3	4.6	0.8
Median	4.6	1.0	5.2	0.3	4.4	0.6
Mean	4.5	0.9	5.6	0.3	4.1	0.6
Sd	0.9	0.2	1.3	0.1	0.8	0.1

GROUP 2

	240 mg Racemic		500 mg -V d-isomer		1000 mg -V d-isomer	
Subject	**Ver**	**Nor**	**Ver**	**Nor**	**Ver**	**Nor**
1	3.2	0.8	3.2	0.3	3.6	0.9
2	5.0	1.0	4.4	0.7	5.5	0.4
3	4.5	0.5	4.2	0.4	7.7	0.7
4	4.4	1.1	4.1	1.0	5.0	1.0
Median	4.4	0.9	4.1	0.6	5.2	0.8
Mean	4.3	0.9	4.0	0.6	5.5	0.8
Sd	0.7	0.3	0.5	0.3	1.5	0.3

240 mg : racemic verapamil; 250, 500, 1000 mg : d-verapamil
ver : verapamil, nor : norverapamil.

2. PHARMACODYNAMICS

2.1) Blood pressure and heart rate:

There were no significant changes in either blood pressure or heart rate in the group receiving the lower doses of d-verapamil. Therefore, the data presented are based on the mean values found in group 2 which contained significant differences from placebo.

a) Supine blood pressure and heart rate:

Supine systolic blood pressure tended to fall after all active treatments but none achieved statistical significance. For example, after 1000 mg d-verapamil (figure 6.1. & 6.3.) supine systolic blood pressure fell from 114 ± 5 (pre-dose) to 104 ± 10 mmHg at 1.5 hours after dosing, compared to the corresponding values of 115 ± 7 and 117 ± 7 mmHg after placebo. Less reduction in supine systolic blood pressure was found after 240 mg racemic or 500 mg d-verapamil.

Supine diastolic blood pressure was also reduced after active treatments and statistical significance was achieved after the 1000 mg d-verapamil and 240 mg racemic verapamil. The average differences between the active treatments and placebo, calculated as an average over the 12 hours, were -7.5 mmHg (95% C.I. -9.4, -5.6) after 1000 mg d-verapamil, -2 mmHg (95% C.I.-0.24,3.6; NS) after 500 mg d-verapamil and -3 mmHg (95% C.I. -4.8, -0.9) after 240 mg racemic verapamil. When the effects on diastolic blood pressure

between active treatments were compared the reduction after 1000 mg d-verapamil was significantly greater than after 240 mg racemic (the difference in average over 12 hours was -4.5 mmHg (95% C.I. -6.6, -2.7)) and also from that after 500 mg d-verapamil (-5.8 mmHg (95% C.I. -7.8, -3.9)).

Supine heart rate increased after verapamil administration and achieved statistical significance after 240 mg racemic, 500 mg d-verapamil and 1000 mg d-verapamil between 1 & 2 hours (figure 6.2. & 6.4.). Between these times the maximum heart rate increases were by 16, 13, 22 bpm after 240 mg racemic, 500 mg and 1000 mg d-verapamil respectively compared to placebo.

b) Erect blood pressure and heart rate:

Erect systolic blood pressure was significantly reduced by verapamil with the greatest reduction occurring after 1000 mg d-verapamil. For erect systolic blood pressure, statistical significance was attained only at 1.5 and 4 hours after the dosing. At 1.5 hour, blood pressure was reduced to 93 $\pm$ 16 mmHg as compared to 115 $\pm$ 7 mmHg with placebo (figure 6.5.). Overall no statistically significant effects were attributable to any of the active treatments although there was a rank order of treatment effect: 240 mg racemic, 500 mg d-verapamil, 1000 mg d-verapamil.

Erect diastolic blood pressure was significantly lowered by all verapamil treatments with the same rank order of treatments. The differences, expressed as the averages over the 12 hour study days, between the active treatments

and placebo, were as follows: -11 mmHg (95% C.I. -14, -8.3) after 1000 mg d-verapamil, -4.5 mmHg (95% C.I. -7, -2) after 240 mg racemic verapamil & -2.9 mmHg (95% C.I. -5.6, -0.35) after 500 mg of d-verapamil. When the effects on erect diastolic blood pressure between active treatments were compared the reduction after 1000 mg d-verapamil was significantly greater than after 240 mg racemic mixture (-6.6 mmHg; 95% C.I. -9.4, -3.7) and also significantly greater than that after 500 mg d-verapamil (-8.2 mmHg; 95% C.I. -11, -5.4).

Erect heart rate was significantly increased after 1 & 2 hours (figure 6.6.) by both the 500 mg and 1000 mg doses of d-verapamil. At one hour after dosing, erect heart rate was increased by 18 bpm after 500 mg and by 20 bpm after 1000 mg d-verapamil, compared to placebo.

2.2) Atrioventricular conduction (PR interval)

PR intervals were significantly increased after all doses of verapamil. The maximum increases occurred between 1 & 2 hours after dosing. Prolongations of about 38 ms in PR interval were achieved one hour after both racemic verapamil and 1000 mg d-verapamil. These were significantly different from placebo (95% CI 12,65, for each treatment). Two hours after dosing the prolongation in PR interval after 240 mg racemic verapamil started to decline towards placebo but the effect of 1000 mg d-verapamil seemed to continue throughout the study day (figure 6.7).

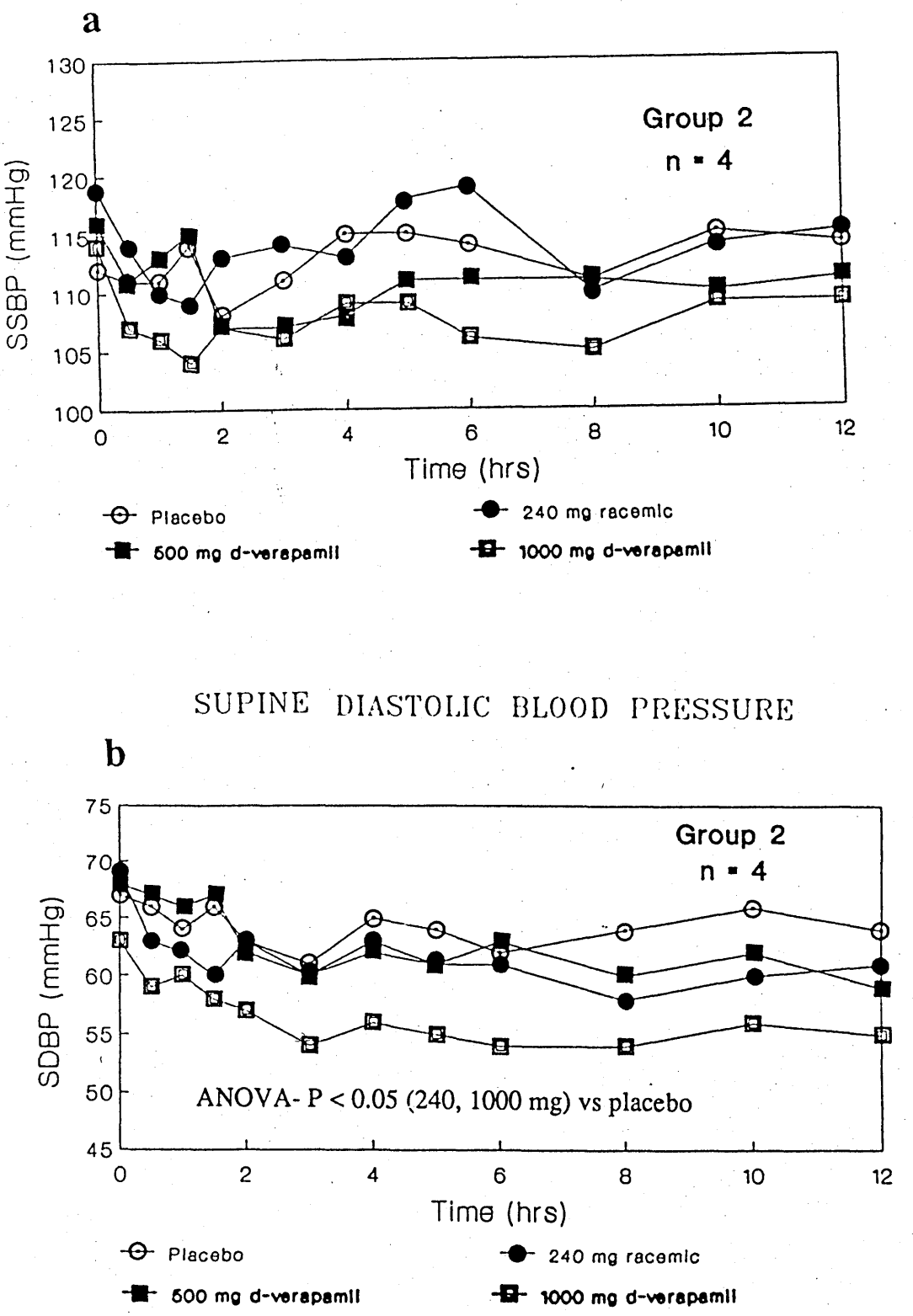
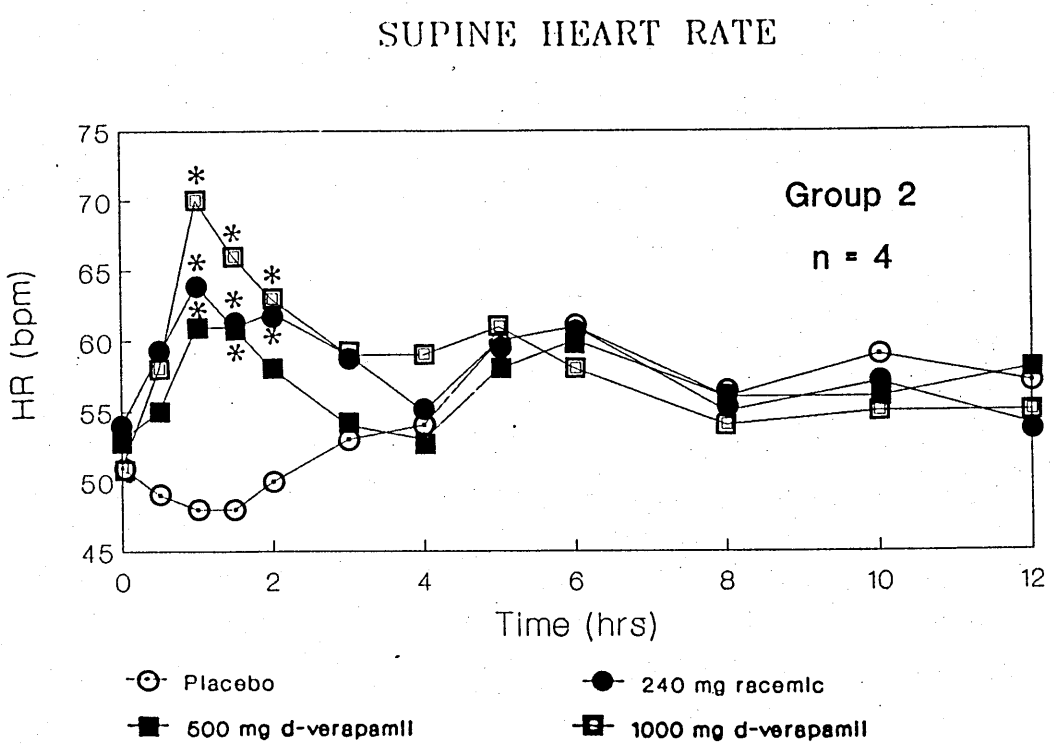


Figure 6.1. Effect of verapamil on supine systolic (a) and diastolic (b) blood pressure.



* Significantly different from placebo
$p < 0.05$ (ANOVA).

Figure 6.2. Effect of verapamil on supine heart rate

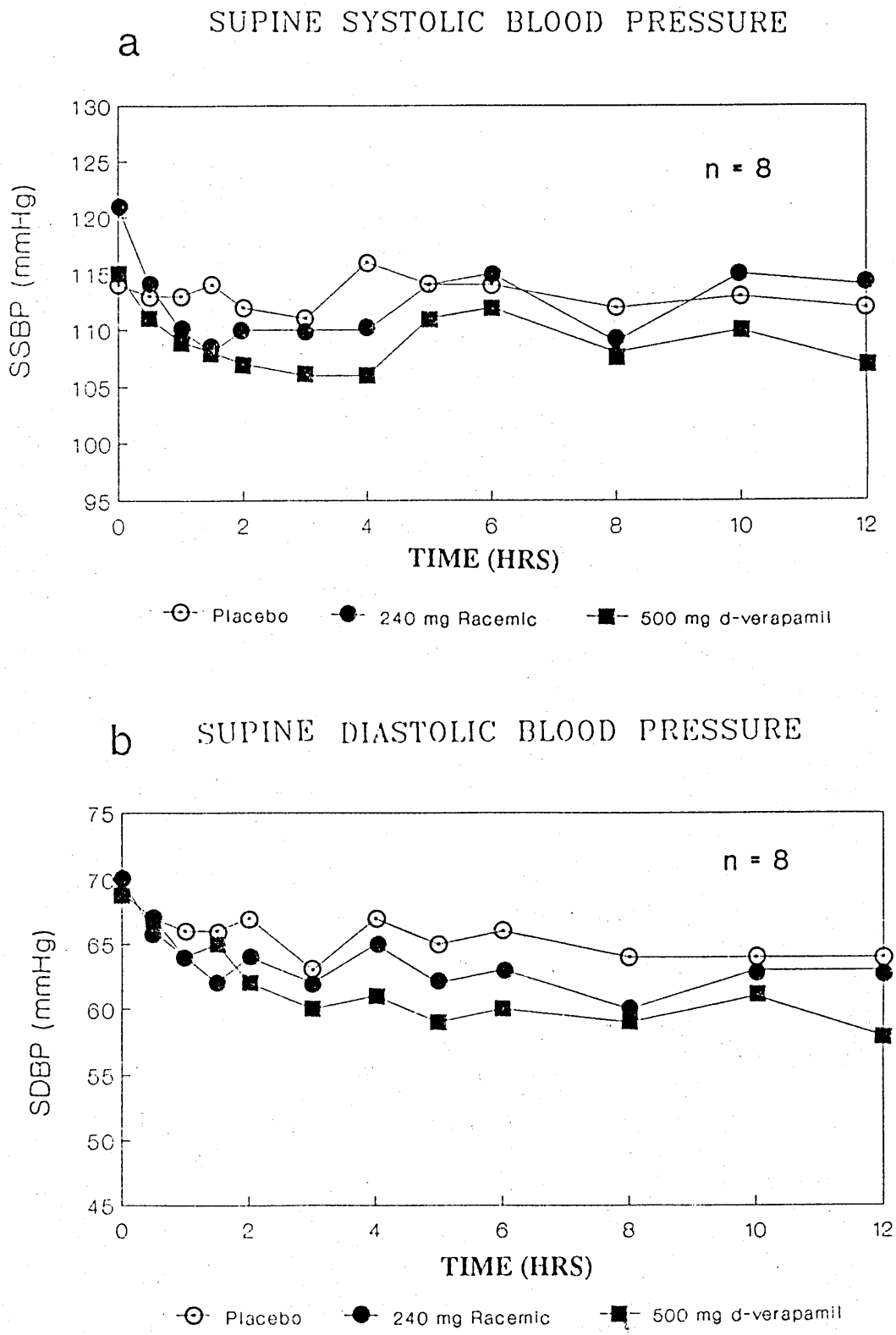
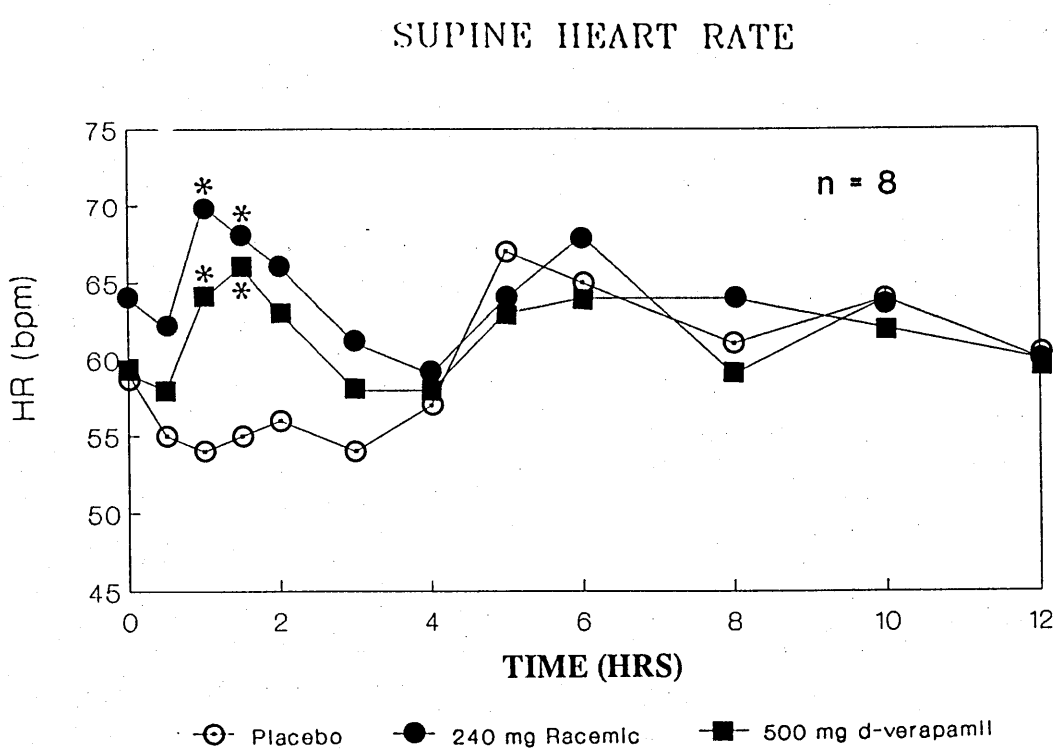
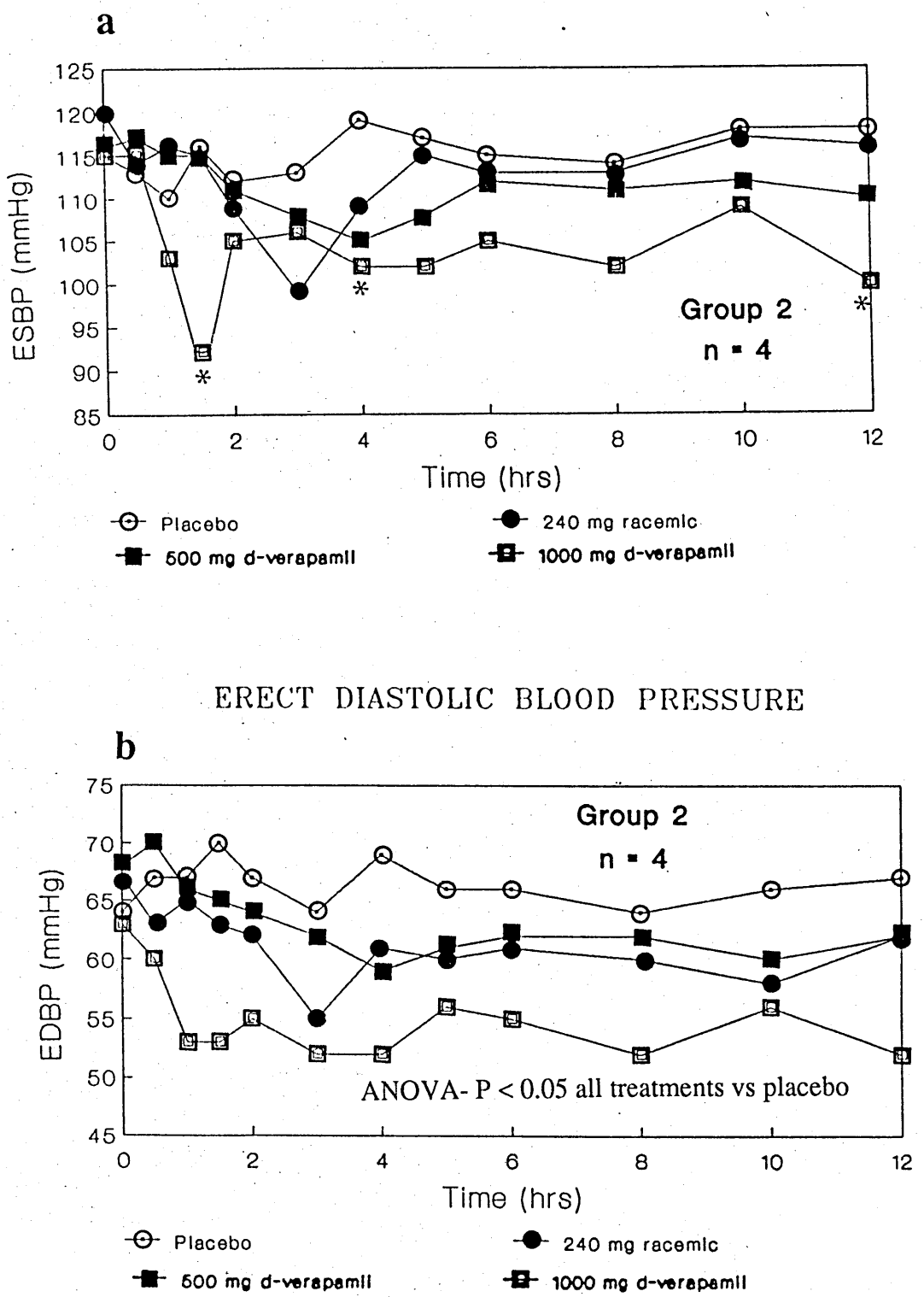


Figure 6.3. Effect of verapamil on supine systolic (a) and diastolic (b) blood pressure for the combined groups.



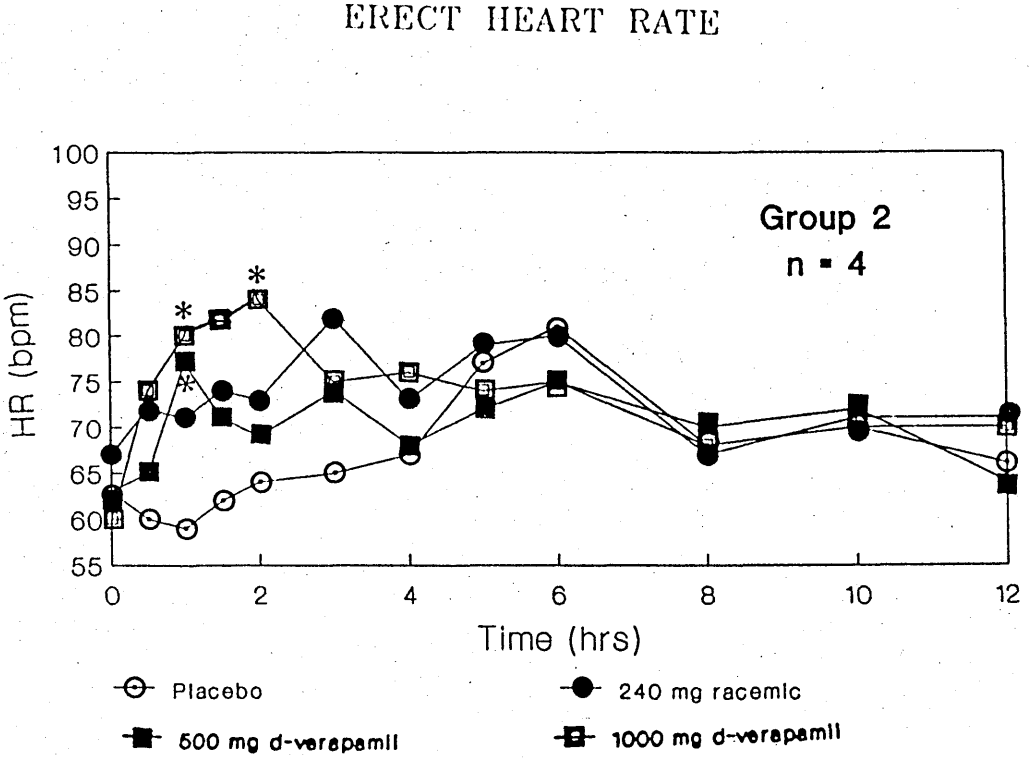
* Significantly different from placebo
$p < 0.05$ (ANOVA).

Figure 6.4. Effect of verapamil on supine heart rate.



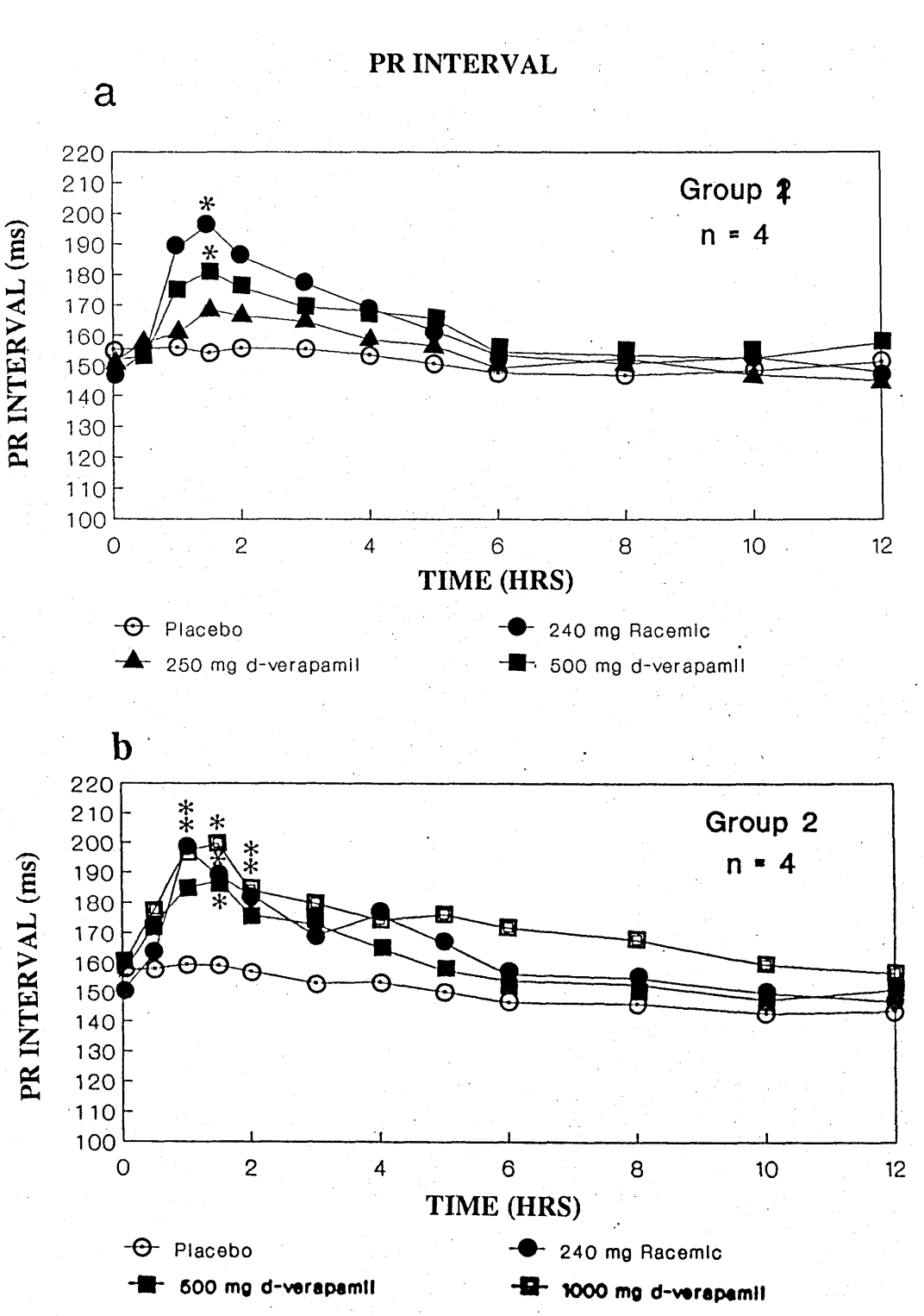
* Significantly different from placebo $p < 0.05$ (ANOVA).

Figure 6.5. Effect of verapamil on erect systolic (a) and diastolic (b) blood pressure.



* Significantly different from placebo
$p < 0.05$ (ANOVA).

Figure 6.6. Effect of verapamil on erect heart rate



* Significantly different from placebo $p < 0.05$ (ANOVA).

Figure 6.7. Effect of verapamil on PR interval in group 1 (a) and group 2 (b).

2.3) Adverse effects

Adverse events are presented in table 6.8. Flushing was most commonly reported and it occurred in 6/8 subjects who received 500 mg d-verapamil and in 4/4 subjects who received 1000 mg d-verapamil. Gastrointestinal upset was the second most frequently reported adverse events with epigastric pain starting 30 minutes after dosing and lasting up to 2 hours reported by all 4 subjects who received 1000 mg d-verapamil. Nausea was reported by 1/8 subjects receiving 500 mg d-verapamil. Symptomatic, postural hypotension was reported by 3 subjects; 1 after 500 mg and 2 after 1000 mg d-verapamil.

3) SIMULATIONS

Using the individual values of the parameters derived from the single dose studies, the predicted concentration time profiles for 500 mg twice daily, 500 mg thrice and four times daily d-verapamil are presented in figures 6.8. and 6.9.

The predicted peak plasma concentrations at steady state fell into the range 1800 - 3000 ng/ml following 500 mg d-verapamil two to four times daily, with associated predicted norverapamil concentration ranges similar to the parent drug. It was also noted that the predicted verapamil and norverapamil concentrations were maintained above 1000 ng/ml (i.e. 2 μM - the "minimal" required level) for about 4 hours or more.

TABLE 6.8.

ADVERSE EFFECTS

Side effect	Placebo	240 mg racemic	500 mg d-verapamil	1000 mg d-verapamil
Flushing	0/8	0/8	6/8	4/4
Nausea/ epigastric pain	0/8	0/8	1/8	4/4
Dizziness/ hypotension	0/8	0/8	1/8	2/4
Headache	0/8	1/8	0/8	0/8

The numbers in the table refer to the proportion of subjects reported side effects to the number of subjects treated.

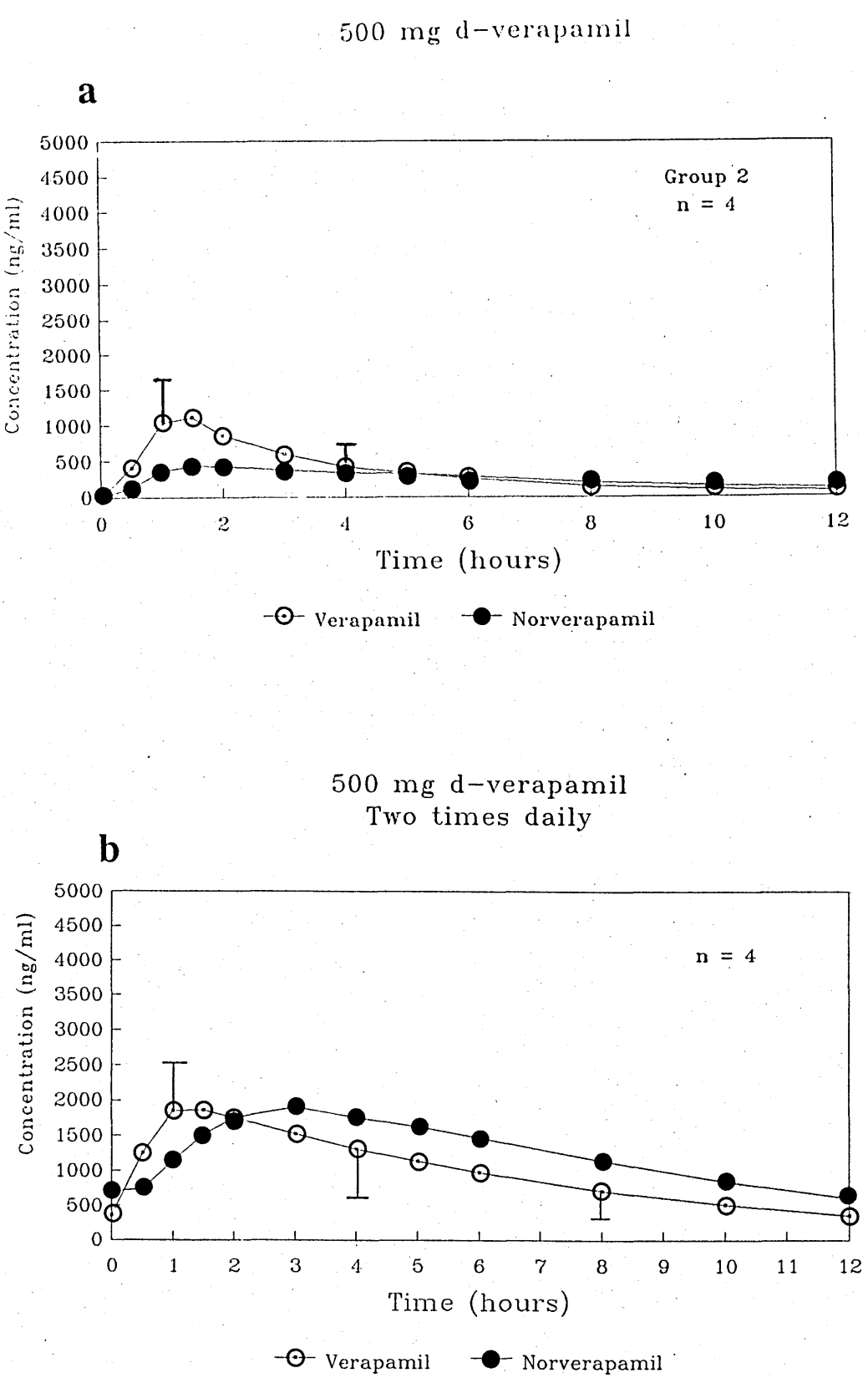


Figure 6.8. a: the plasma concentration of d-verapamil after 500 mg single dose; b: predicted steady state concentration after 500 mg twice daily.

Error bars: SD,

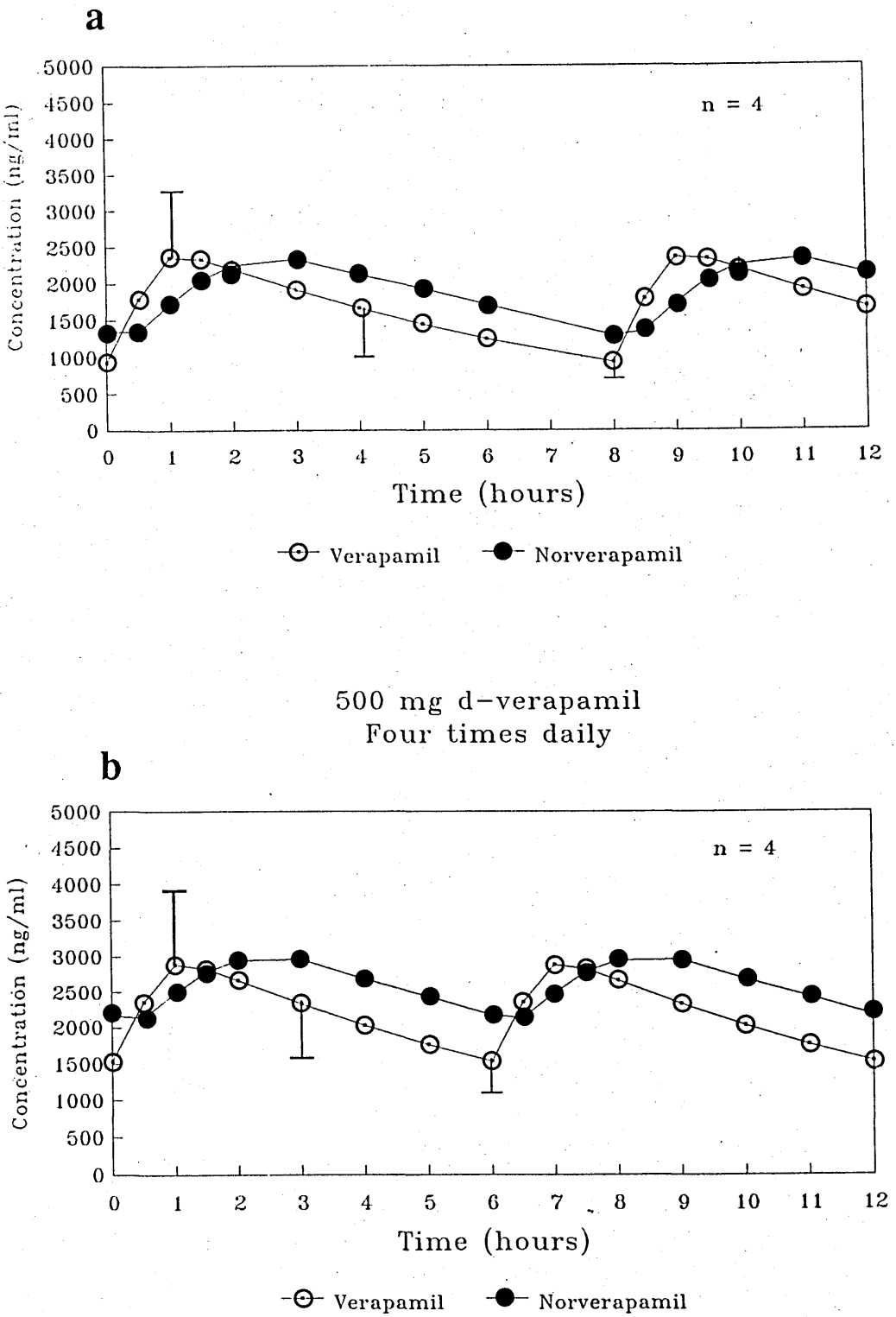


Figure 6.9. Predicted steady plasma concentration from single dose data after 500 mg three times daily (a) and four times daily (b).

Error bars: SD.

DISCUSSION

With respect to the use of single isomer d-verapamil as an adjuvant cytotoxic drug treatment the principal aims of this study were to determine the disposition and cardiovascular effects of d-verapamil when administered in doses likely to produce the required (or thought to be required) plasma drug concentrations.

The target range of 1000-3000 ng/ml (2 - 6 μM) can be reached with the intravenous administration of racemic verapamil (9 micrograms/kg/min) but in association with a severe and unacceptable degree of cardiac toxicity (Ozols et al., 1987).

In this single dose study, 1000 mg d-verapamil produced peak concentrations around 2000 ng/ml (about 4 μM). However, this peak level was relatively short-lived and declined 0.5 to 1 hour after dosing. It seems likely that the optimal cytotoxic adjuvant will require that the verapamil level should be sustained for a longer period. In addition, although the 1000 mg dose of d-verapamil was associated with prolongations of PR interval which were not significantly different from that associated with 240 mg of racemic verapamil, none of the subjects had any degree of A-V block. However, significant falls in blood pressure, particularly on standing, occurred with 1000 mg at approximately the time of maximum drug concentrations. From the effects on blood pressure and A-V conduction it appears that d-verapamil had a relatively greater effect on lowering blood pressure than on increasing A-V conduction. Thus, considering d-verapamil

as an adjuvant in the treatment of cancer, the hypotensive action may be potentially more important than the negative dromotropic effect. A further consideration for the relative importance of these effects is that there are reports that the effect of verapamil on A-V conduction is decreased with age (Abernethy et al., 1986).

The reported side effects, especially with the 500 and 1000 mg doses, included flushing, hypotension and epigastric pain. The epigastric pain may be due to a local gastric irritant effect because of the bulk of the tablets used (200 mg x 5). There were no clinical signs indicating an impairment of myocardial contractility (negative inotropic effect).

In the present study, we used the concept of pharmacokinetic modelling to extrapolate beyond the existing data (Colburn et al., 1987) to predict steady state plasma concentrations from single dose data. The predicted concentrations were adjusted to take account of the presumed accumulation of verapamil at steady state on the assumption that d-verapamil displayed the same changes in disposition as racemic verapamil during continued administration (Shand et al, 1981; Freedman et al., 1981). On the basis of these predictions, a regimen of 500 mg d-verapamil four times daily would result in a peak plasma level of 3000 ng/ml (about 6 μM).

It appears, therefore, that relatively high concentrations, comparable to those indicated by the <u>in vitro</u> studies, can be achieved after the administration of

the d-isomer of verapamil with little serious cardiac toxicity.

It is not yet clear if the effective range of verapamil concentrations obtained *in vitro* is directly applicable to man since many *in vivo* factors might intervene to affect the availability of verapamil at the required site of action. These include:

1. Verapamil in plasma is bound to plasma albumin (60%) and to alpha1-acid glycoprotein (McGowan et al., 1983) which is increased in malignancy (Snyder & Ashwell, 1971; Weiss et al., 1979; Chio et al., 1979; for review see Wood, 1986). Potentially, therefore, the free concentration of verapamil, which can more readily penetrate into the tissues, will be reduced.

2. Drug concentrations in plasma do not necessarily reflect the concentrations in tissues and this may be particularly true of neoplastic tissues.

3. Norverapamil, which is generated as a result of the metabolism of verapamil in the liver, reaches concentrations which have been shown *in vitro* to have additive adjuvant effects. Thus, when verapamil and norverapamil are used in relatively low concentrations, each of 2 μM, the cytotoxic effect is maximal (Merry et al., 1989). If this translates to *in vivo* effects then d-verapamil can be administered in a flexible range of doses to generate norverapamil in reasonable concentrations to achieve the desired combined concentrations. Thus a total concentration of 2 μM (1000 ng/ml) for the drug and metabolite might be achieved at

relatively low doses of d-verapamil with a further reduction in cardiovascular side effects. Norverapamil appears to have only approximately 20% of the coronary vasodilatory activity of the parent compound (Neugebauer et al., 1978) and is itself unlikely to have major haemodynamic effects for the range of concentrations required.

4. Since verapamil may inhibit hepatic enzymes activity (c.f. its own clearance) and since there is evidence that it inhibits the metabolism of other drugs, such as antipyrine (Bauer et al., 1986), an interaction between verapamil and a concurrently administered chemotherapeutic agent may result in higher plasma levels and probably enhanced cytotoxic effects. Not only might this result in more chemotherapeutic toxicity but if the chemotherapeutic agent was also cardiotoxic (e.g. adriamycin) there would be further reasons for concern about the combined use.

It is concluded that d-verapamil can be used instead of racemic verapamil to achieve a range of concentrations thought to be appropriate for adjuvant cancer treatment but without serious cardiovascular side effects. However, the optimal dosing regimen and the interactions with cytotoxic drugs and the impact of the disease state require further clinical study in cancer patients.

CHAPTER SEVEN

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF NICARDIPINE IN PATIENTS WITH RENAL IMPAIRMENT

INTRODUCTION

Since calcium antagonist drugs are extensively metabolised by the liver with a plasma clearance which depends primarily on liver blood flow and on hepatocellular function, many studies have concentrated upon investigations of the effect of liver disease on drug disposition and dose requirements. Such studies have shown that there were significant reductions in the plasma clearance of verapamil (Somogyi et al., 1981), nifedipine (Kleinbloesem et al., 1986), nisoldipine (Van Harten et al., 1987; Joeres et al., 1987) and nimodipine (Gengo et al., 1987) in patients with hepatic impairment. Little attention, therefore, has been addressed to the effects of renal impairment but since cardiovascular disease and renal impairment frequently coincide, it is of comparable importance to investigate the impact of renal disease on the clinical pharmacology of calcium antagonist drugs.

The present study investigates the pharmacokinetics and pharmacodynamics of nicardipine in patients with varying degrees of renal impairment.

SUBJECTS AND METHODS

The study design was single blind (patient-blind) and randomised. Three separate groups of patients whose clinical characteristics are listed in table 7.1. were recruited for the study.

Group 1: patients with mild to moderate essential hypertension with normal renal functions;

group 2: patients with mild to moderate essential hypertension with varying degrees of renal impairment;

group 3: patients with end-stage renal failure on regular haemodialysis three times per week (study days occurred between the dialysis treatments).

All patients gave written, informed consent and reported to the Clinical Pharmacology Research Unit having fasted over night. The 3 groups were randomly assigned to receive a single intravenous dose and a single oral dose (acute) of nicardipine on two separate study days with a wash out period of 7 days between. Groups 1 and 2 additionally undertook a third study day following a 1 week of treatment with nicardipine 45 mg twice daily. Intravenous nicardipine was administered as a short intravenous infusion (70 ug/kg body weight) over 10 minutes. Blood samples were collected before the dose and at 2, 5 and 10 minutes during infusion and 2, 5, 10, 20, 40, 60 minutes and 2, 3, 4, 5, 6, 8 hours after infusion.

Oral nicardipine was administered as a slow release formulation (45 mg, biphasic formulation). The morning dose was given at 8:30 with 150 mls of water. Blood samples were collected before the dose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 12 hours after the dose.

TABLE 7.1.

CLINICAL CHARACTERISTIC OF PATIENTS

Group	CRCL ml/min	Age (years)	Weight (Kg)	Previous Medication
Normal renal function				
1.1	72	53	57	
1.3	97	61	90	*Bendrofluazide
1.4	89	47	55	
1.5	88	64	85	*Allopurinol
1.6	103	58	80	
1.7	95	41	63	
Mean	91±11	54±9	72±15	
Renal Impairment group				
2.1	40	47	75	
2.2	55	57	78	Spironolactone
2.3	43	65	55	Diltiazem, *Bendrofluazide
2.4	49	50	65	Nifedipine
2.5	49	62	105	*Bendrofluazide, KCL, aspirin
2.6	22	65	69	*Frusemide
2.7	13	62	77	*Frusemide
Mean	39±16	58±7	75±16	
Dialysis group				
3.1		50	65	Ferrous sulphate
3.2		33	47	Ferrous sulphate
3.3		49	77	
3.4		20	66	
3.5		58	67	*Dipyridamole
3.6		23	47	Ferrous sulphate
3.7		61	75	
3.8		30	73	Floxacillin
Mean		41±16	65±12	

CRCL : Creatinine clearance.

* Drugs which are continued throughout the study

Dialysis group (group 3) were continued on vitamin B, ascorbic acid, folic acid, Alu-Cap (Aluminium hydroxide) throughout the study.

Blood pressure and heart rate: were recorded in duplicate by a semiautomatic shygmomanometer (Sentron, Bard Medical) at times corresponding to the times of blood sampling for drugs.

Apparent Liver blood flow: was measured 1 hour after dosing from the plasma clearance of an intravenously administered dose of indocyanine green (ICG) (0.5 mg/kg).

Renal function: glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured 1.5 hours after dosing from the clearance of ^{51}Cr-EDTA and ^{125}I-Hippuran respectively.

Analytical Methods

Nicardipine plasma levels were determined by an HPLC method (Wu et al., 1984)

Indocyanine green plasma levels were determined by an HPLC method as described by Rappaport et al., (1982).

Pharmacokinetic analysis

The individual concentration time profiles were first plotted on semi-log graph. The concentrations after intravenous infusion were best fitted to a two compartment model of the form described by Gibaldi & Perrier (1975) as described in the methods chapter.

The plasma drug clearance and volume of distribution were directly estimated from the model. Elimination half life was calculated by dividing $0.693/\beta$.

After oral administration, the concentration time profile was biphasic in shape and it was inappropriate to fit the data to a model. The pharmacokinetic parameters such

as C_{max} or t_{max} and bioavailability were estimated by standard technique as described in the method section.

Statistical Analysis

Logarithmic (to the base e) transformation was required to normalise the distribution of plasma clearance of nicardipine and for liver blood flow data. For comparison between groups, one way analysis of variance (ANOVA) was used. The relationship between the pharmacokinetic parameters and indices of renal function for the whole group was undertaken by linear regression analysis. Time and treatment effect on blood pressure and heart rate was evaluated by repeated measures analysis of variance using BMDP package. Data throughout are presented as mean $\pm$ SD.

RESULTS

Intravenous infusion

Mean plasma concentration time profiles of nicardipine for the 3 groups are shown in figure 7.1.. The fitted pharmacokinetic parameters are presented in table 7.2. and the derived pharmacokinetic parameters in table 7.3.

The plasma clearance of nicardipine was significantly lower in patients with renal impairment (group 2) compared to those with normal renal function (group 1): (10.41 vs 6.53 ml/min/kg; P = 0.03), whereas plasma clearance in patients with end stage renal failure (group 3) (12.54 ml/min/kg) was not different from the value in the normal group (figure 7.2.). These changes were associated with a

slight and insignificant increase in elimination half life in group 2 & 3 compared to group 1. The volume of distribution of the central (Vc) and the peripheral compartment, although apparently higher in group 3, was not significantly different from group 1 or group 2 (figure 7.3.).

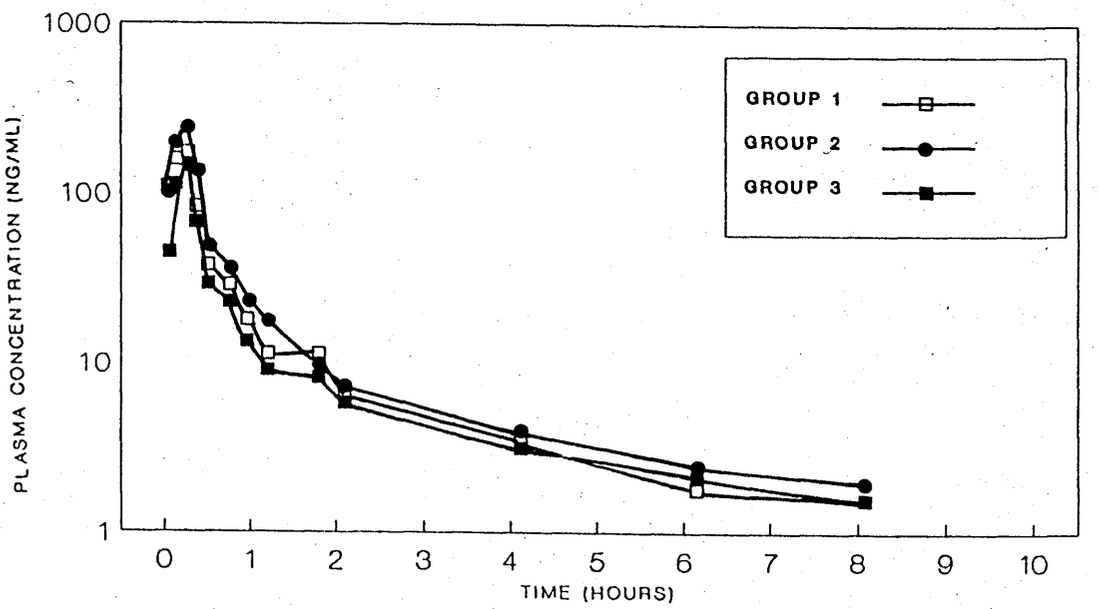


Figure 7.1. Mean plasma nicardipine levels following an intravenous infusion of nicardipine HCL (70 ug/kg for 10 minutes). The plasma concentrations decline in bi-exponential manner.

TABLE 7.2.

THE PHARMACOKINETIC PARAMETERS OF NICARDIPINE
FOLLOWING INTRAVENOUS INFUSION 70 UG/KG OVER 10 MINUTES

Group	A (ug/l)	Alpha (1/h)	B (ug/l)	Beta (1/h)	R^2 value
GROUP 1					
1.1	0.014	14.9	0.0138	0.400	0.904
1.3	0.0032	50.0	0.0189	0.356	0.801
1.4	0.0056	11.2	0.0131	0.450	0.942
1.5	0.0062	13.3	0.0160	0.500	0.986
1.6	0.0054	16.0	0.0226	0.600	0.850
1.7	0.0058	7.0	0.0180	1.680	0.877
Mean	0.0067	18.73	0.0171	0.664	
Sd	0.0037	15.64	0.0035	0.505	
GROUP 2					
2.1	0.0105	16.4	0.0230	0.250	0.902
2.2	0.0122	3.6	0.0106	0.290	0.970
2.3	0.0125	40.0	0.0770	0.360	0.912
2.4	0.0121	15.8	0.0240	0.500	0.976
2.5	0.0046	21.5	0.0112	0.877	0.956
2.6	0.0140	22.8	0.0312	0.409	0.960
2.7	0.0154	9.7	0.0416	0.135	0.988
Mean	0.0116	18.5	0.0312	0.403	
Sd	0.0035	11.6	0.0230	0.240	
GROUP 3					
3.2	0.0066	6.6	0.0300	0.079	0.823
3.3	0.0074	23.9	0.0090	1.120	0.893
3.4	0.0179	30.0	0.0083	0.395	0.986
3.5	0.0084	16.0	0.0140	1.170	0.992
3.6	0.0065	16.5	0.0164	0.691	0.994
3.7	0.0046	8.9	0.0240	0.293	0.946
3.8	0.0072	8.5	0.0036	0.434	0.942
Mean	0.0084	15.8	0.0150	0.590	
Sd	0.0043	8.7	0.0093	0.410	

R^2: % variability explained by the model.

TABLE 7.3.

THE DERIVED PHARMACOKINETIC PARAMETERS OF NICARDIPINE FOLLOWING INTRAVENOUS INFUSION OF 70 UG/KG OVER 10 MINUTES

GROUP 1

Group	K21 (1/h)	Cl (ml/min/kg)	V1 (l/kg)	T1/2 Alpha (h)	T1/2 Beta (h)
1.1	0.77	10.49	0.081	0.046	1.73
1.3	2.34	8.37	0.065	0.014	1.95
1.4	1.37	16.21	0.260	0.062	1.54
1.5	1.63	8.82	0.130	0.052	1.39
1.6	2.68	7.42	0.120	0.043	1.16
1.7	6.35	11.13	0.036	0.010	0.41
Mean	2.52	10.41	0.115	0.037	1.36
Sd	1.99	3.15	0.079	0.020	0.54

GROUP 2

2.1	0.77	6.66	0.075	0.042	2.77
2.2	0.50	9.40	0.270	0.191	2.39
2.3	2.45	3.36	0.034	0.017	1.93
2.4	1.39	7.15	0.075	0.044	1.39
2.5	2.75	10.06	0.088	0.032	0.79
2.6	1.27	5.33	0.043	0.030	1.69
2.7	0.48	3.78	0.083	0.071	5.13
Mean	1.37	6.53	0.096	0.061	2.29
Sd	0.91	2.58	0.079	0.060	1.41

GROUP 3

3.2	0.415	9.68	0.46	0.105	8.770
3.3	2.350	13.18	0.07	0.029	0.617
3.4	0.574	9.64	0.03	0.023	1.750
3.5	2.780	11.09	0.09	0.043	0.590
3.6	2.190	15.46	0.17	0.042	1.000
3.7	1.560	7.68	0.27	0.077	2.370
3.8	0.636	21.11	0.22	0.082	1.600
Mean	1.500	12.54	0.18	0.057	2.380
Sd	0.960	4.56	0.15	0.030	2.880

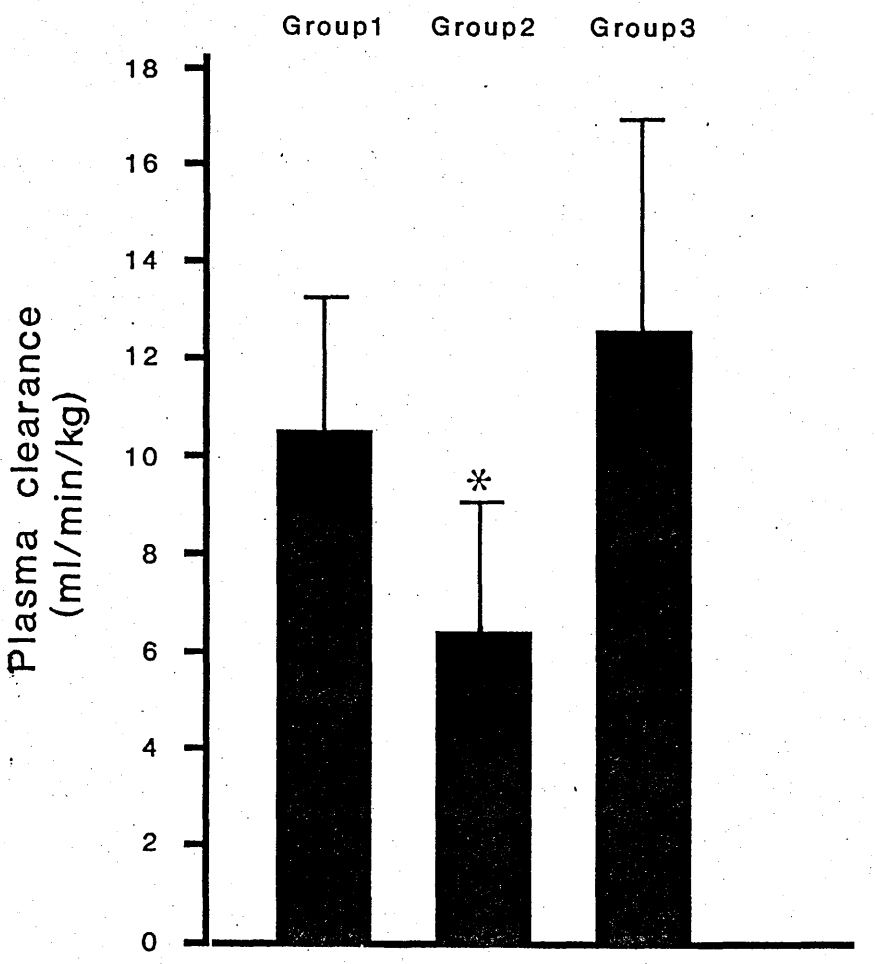


Figure 7.2. The plasma clearance of nicardipine following acute intravenous infusion of 70 ug/kg over 10 minutes.

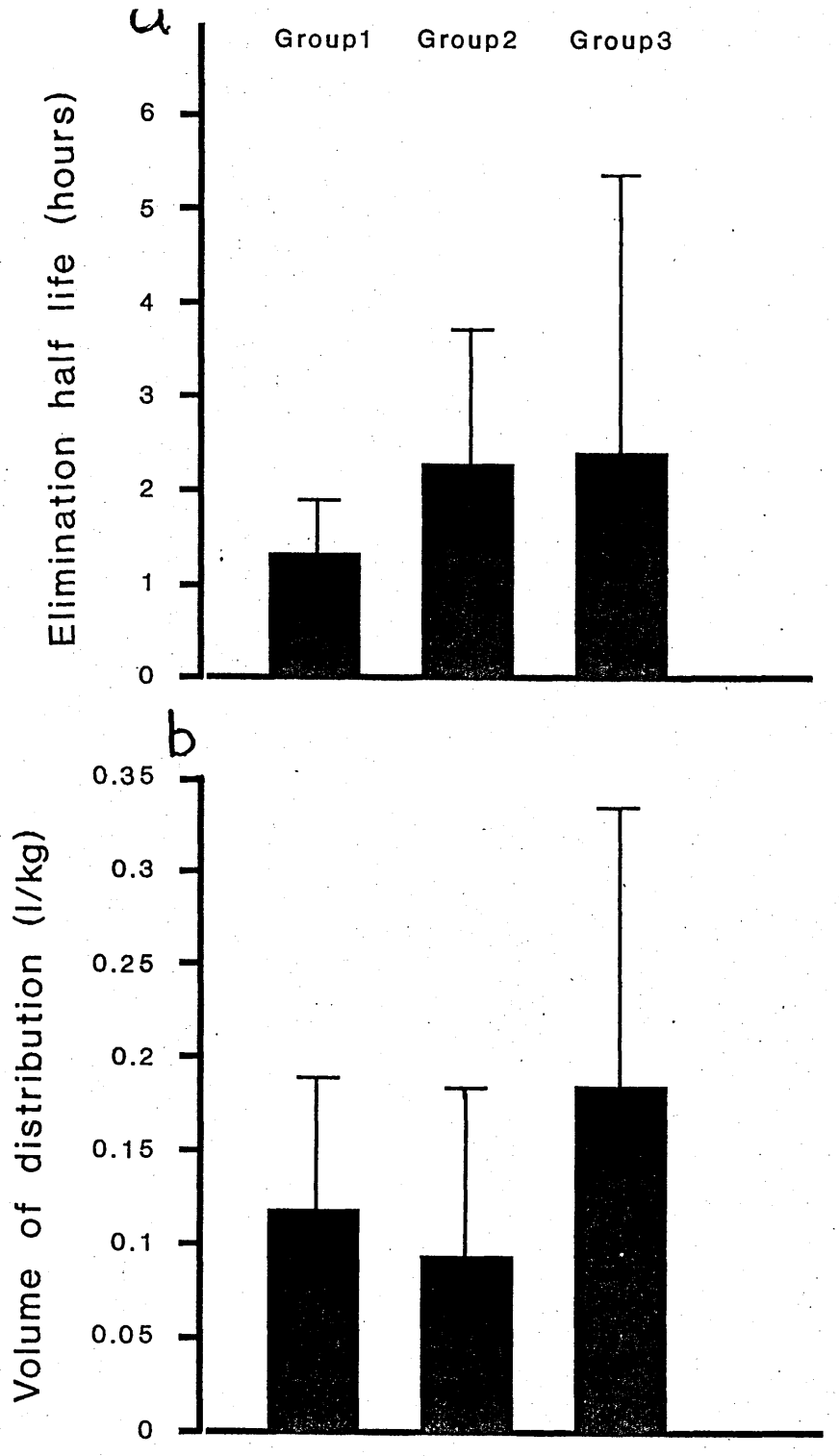


Figure 7.3. Elimination half life (a) and volume of distribution (b) of nicardipine following acute intravenous infusion of 70 ug/kg over 10 minutes

Single oral doses of nicardipine

Mean plasma concentration time profiles after the single oral doses of nicardipine are presented in figure 7.4. The pharmacokinetic parameters are presented in table 7.4. and figure 7.5.

The $AUC_{0-\infty}$ after the first dose was significantly higher in patients with renal impairment (group 2) compared to patients with normal renal function (group1) (153 vs 249 ng/ml*h; $P < 0.05$). The AUC in the dialysis group (group 3) was 184 ng/ml*h which is not significantly different from group 1. Correspondingly, Cl/F was decreased from 105 ml/min/kg in patients with normal renal function to 50 ml/min/kg; $P < 0.05$, in patient with renal impairment but it was 93 ml/min/kg in the dialysis group. Peak plasma level was slightly higher in patients with renal impairment (group 2) compared to group 1 (30 vs 51 ng/ml) but this was not statistically significant. Peak plasma level was 33 ng/ml in the dialysis group.

Chronic oral doses of nicardipine

The derived pharmacokinetic parameters of nicardipine after chronic treatment are presented in table 7.5. and figure 7.5. During continued administration the $AUC_{0-12\ h}$ was significantly higher in patients with renal impairment (group 2) compared to patients with normal renal function (group 1) (194 vs 509 ng/ml*h; $P = 0.006$) and the clearance/F was significantly lower in patients with renal impairment compared to normal renal function group (20.8 vs 70.2 ml/min/kg; $P = 0.006$). Peak plasma level (C_{max})

achieved a level of 87 ng/ml in the renal impairment group which is significantly higher than 38.3 ng/ml in group 1; P = 0.01. During the translation from acute to chronic, clearance/F was reduced with chronic dosing from 105.4 to 70.2 ml/min/kg (33%) in group 1 which is not significant whereas in group 2 clearance/F was significantly decreased from 50 to 21 ml/min/kg (58%) (P = 0.005) and the C_{max} increased from 51 to 87 ng/ml (70%) (P = 0.006) (figure 7.5.).

The bioavailability after single doses of nicardipine was about 15% and there were no significant differences between the three groups. During chronic dosing there was a significant difference between the bioavailability in group 1 (18%) and group 2 (34%) (P < 0.02) and, in group 2, the bioavailability had significantly increased from 18% after acute dosing to 34% after chronic dosing (mean difference 16%, P < 0.003).

Apparent liver blood flow

There were no significant differences in liver blood flow between the groups and there were no effects attributable to nicardipine (table 7.6.).

Renal functions

Patients with renal impairment had significantly lower ERPF and GFR compared to patients with normal renal function and there were no effects attributable to nicardipine. (table 7.7. & 7.8.)

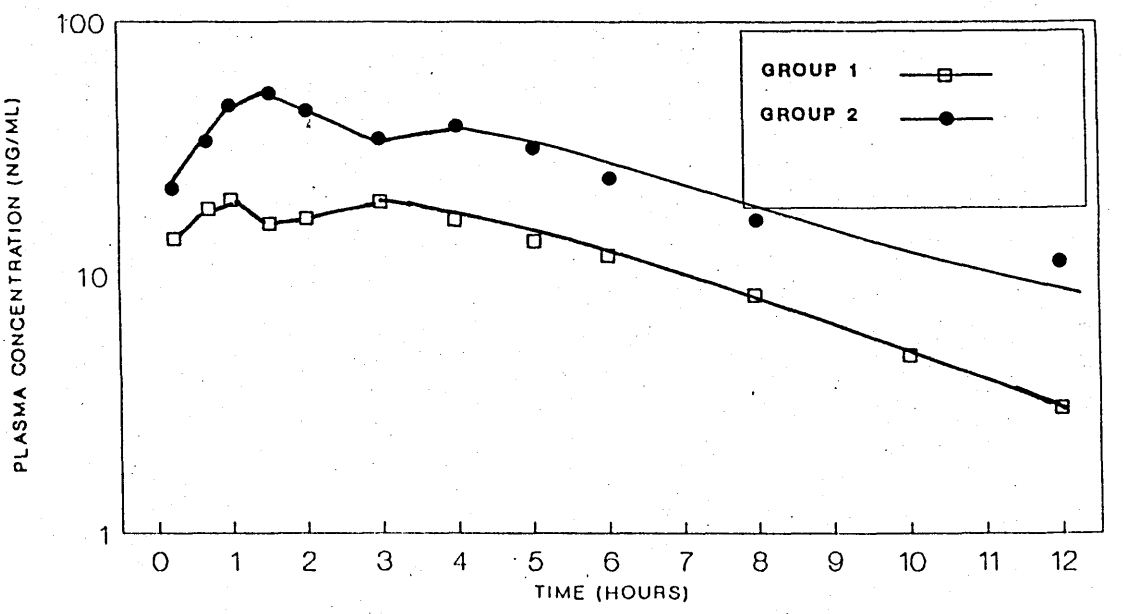


Figure 7.4. Mean plasma nicardipine levels for (group 1) and (group 2). The plasma levels shows the biphasic pattern of absorption between 1 and 3 hours.

TABLE 7.4.

THE PHARMACOKINETIC PARAMETERS OF NICARDIPINE FOLLOWING SINGLE ORAL DOSES (45 MG SR)

Group	AUC (ng/ml*h)	C_{max} (ng/ml)	T_{max} (h)	Cl/F (ml/min/kg)	Bioavailability (%)
GROUP 1					
1.1	282.3	53.2	5.0	46.6	23.1
1.3	203.2	46.7	0.7	41.0	25.1
1.4	54.5	8.4	5.0	250.0	6.6
1.5	191.4	31.1	5.0	46.0	21.1
1.6	113.1	22.3	5.0	82.9	9.0
1.7	71.8	19.9	4.0	165.9	6.4
Mean	152.7	30.26	4.1	105.4	15.2
Sd	87.9	17.00	1.7	85.1	8.8
GROUP 2					
2.1	301.7	75.5	2.2	33.1	22.5
2.2	140.0	26.6	1.5	86.7	14.2
2.3	389.0	97.3	0.7	35.0	16.6
2.4	154.8	24.6	6.0	74.5	10.7
2.5	142.8	28.2	1.5	50.0	20.6
2.6	390.0	63.1	3.2	27.9	25.0
2.7	226.8	42.4	4.0	42.9	13.5
Mean	249.3	51.1	2.7	50.0	17.6
Sd	111.6	28.2	1.8	22.3	5.2
GROUP 3					
3.1	69.2	21.4	5.0	166.7	NR
3.2	155.6	18.1	6.0	102.6	10.0
3.3	95.0	23.8	1.5	102.5	14.2
3.4	135.0	38.7	0.9	84.2	13.4
3.5	425.5	74.3	3.2	26.3	40.4
3.6	396.0	56.8	5.0	40.3	31.0
3.7	70.2	14.6	2.2	142.4	5.2
3.8	124.4	19.6	0.9	82.6	24.5
Mean	183.8	33.4	3.1	93.4	19.8
Sd	143.5	21.6	2.0	47.0	12.6

TABLE 7.5.

THE PHARMACOKINETIC PARAMETERS OF NICARDIPINE FOLLOWING CHRONIC ORAL DOSING (45 MG SR B.D)

GROUP 1

Group	AUC (ng/ml*h)	C_{max} (ng/ml)	T_{max} (h)	Cl/F (ml/min/kg)	Bioavailability (%)
1.1	484.0	92.2	3.17	25.2	36.9
1.3	192.0	40.2	3.17	41.2	20.9
1.4	105.0	15.8	0.92	119.0	13.7
1.5	172.0	29.0	3.17	47.8	18.3
1.6	109.0	24.6	4.00	80.0	8.8
1.7	101.0	27.8	0.67	108.0	8.4
Mean	193.8	38.3	2.50	70.2	17.8
Sd	147.2	27.6	1.40	38.1	10.6

GROUP 2

2.1	643.0	118.0	0.67	14.3	36.9
2.2	254.0	62.4	1.50	35.4	25.8
2.3	533.0	97.0	1.50	23.9	18.9
2.4	601.0	89.0	0.92	18.1	39.2
2.5	302.0	48.9	3.17	22.1	40.1
2.6	772.0	121.0	2.17	12.7	50.1
2.7	457.0	70.5	4.00	19.5	26.4
Mean	509.0	87.0	1.99	20.8	33.9
Sd	186.0	27.6	1.21	7.6	10.7

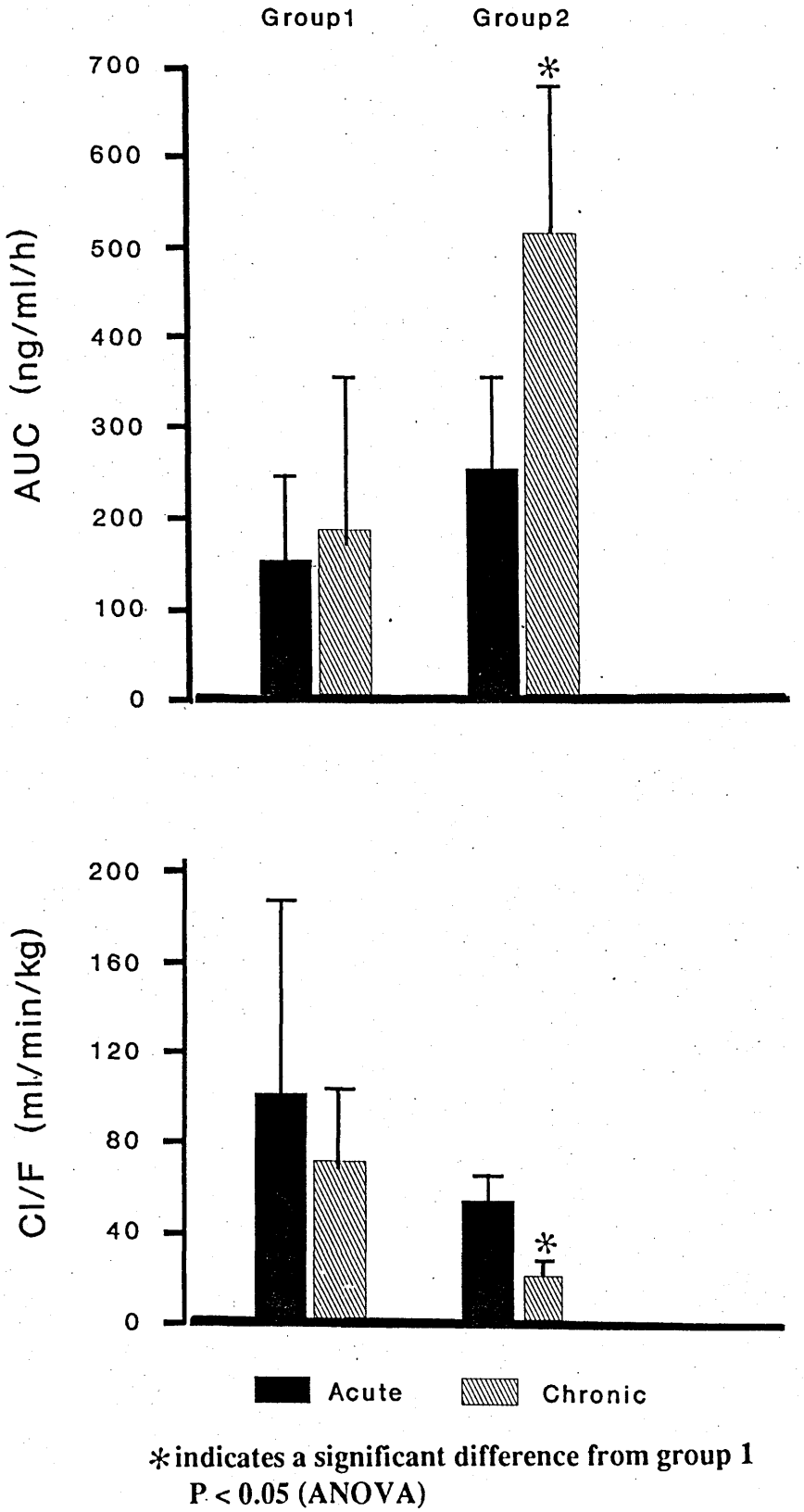


Figure 7.5. The area under the concentration time curve (AUC) and Cl/F following oral dosing with nicardipine.

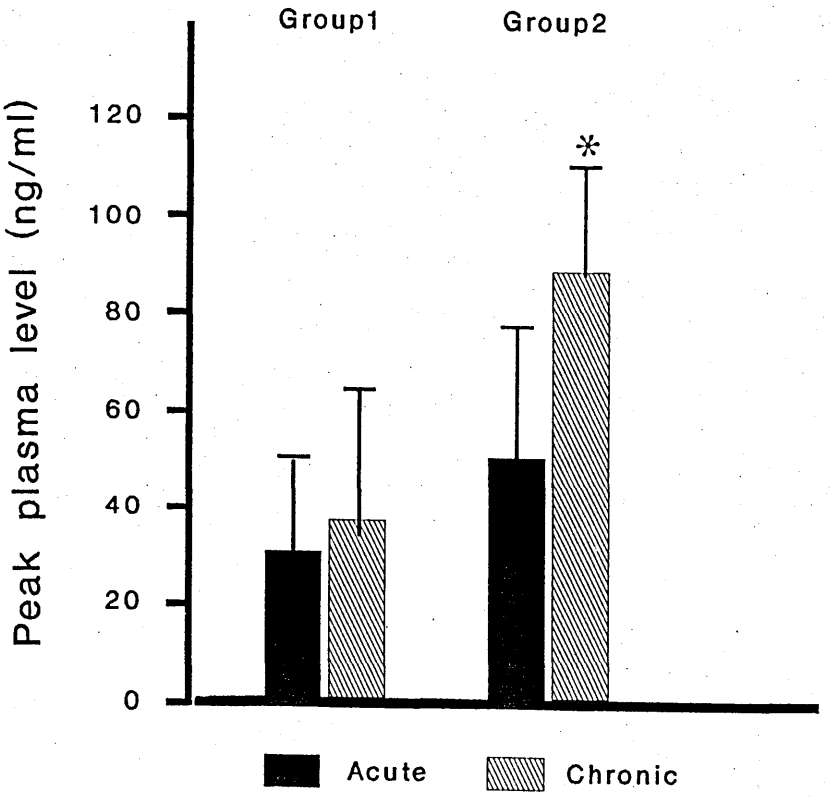


Figure 7.6. Peak plasma level (C_{max}) following acute and chronic oral dosing of nicardipine.

TABLE 7.6.

LIVER BLOOD FLOW FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF NICARDIPINE HCL

	Liver blood flow (ml/min/kg)		
Pat. No.	**I.V.**	**Acute**	**Chronic**
Group 1			
1:1	23.3	17.0	26.9
1:3	37.6	43.4	28.4
1:4	21.2	31.1	22.9
1:5	13.8	13.8	14.5
1:6	16.6	17.5	15.2
1:7	25.1	25.7	19.7
Mean	22.9	24.7	21.3
Sd	8.3	11.1	5.8
Group 2			
2:1	32.6	22.4	32.6
2:2	13.0	16.6	14.2
2:3	14.1	15.3	15.3
2:4	15.7	NR	19.2
2:5	NR	12.0	12.4
2:6	61.9	15.4	42.5
2:7	8.8	11.0	12.3
Mean	24.3	15.4	21.2
Sd	20.1	4.0	11.7

NR : no result.

TABLE 7.7.

EFFECTIVE RENAL PLASMA FLOWS (ERPF) FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF NICARDIPINE HCL

	ERPF (ml/min)		
Pat. No.	**I.V.**	**Acute**	**Chronic**
Group 1			
1:1	640	399	350
1:3	448	485	417*
1:4	431	430	390
1:5	398	431	389
1:6	413*	507*	410*
1:7	680	606	481
Mean	502	466	406
Sd	124	90	43
Group 2			
2:1	129	116	163
2:2	321	303	198
2:3	NR	NR	NR
2:4	270	335	382
2:5	236	245	206
2:6	87	78	67
2:7	NR	47	48
Mean	209	187	178
Sd	98	122	120

All ERPF values have been normalised to 1.73 m^2 Body Surface Area.

NR = No Result
* = Value calculated on the basis of plasma data extrapolated to infinity because urine collection was incomplete.

TABLE 7.8.

GLOMERULAR FILTRATION RATE (GFR) FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF NICARDIPINE HCL

	GFR (ml/min)		
Pat. No.	**I.V.**	**Acute**	**Chronic**
Group 1			
1:1	88.2	90.6	69.7
1:3	83.4	86.9	86.9*
1:4	91.7	93.8	86.5
1:5	72.4	82.1	68.0
1:6	80.4*	88.2*	95.2*
1:7	107.0	115.0	116.0
Mean	87.0	92.7	87.0
Sd	12.0	11.6	17.7
Group 2			
2:1	33.1	35.0	37.8
2:2	72.5	58.9	38.9
2:3	NR	NR	NR
2:4	71.4	57.3	71.4
2:5	68.9	71.3	64.1
2:5	22.2	17.4	12.6
2:6	NR	8.9	7.9
Mean	53.6	41.3	38.8
Sd	24.0	24.9	25.9

All GFR values have been normalised to 1.73 m^2 Body Surface Area.

NR = No Result
* = Value calculated on the basis of plasma data extrapolated to infinity because urine collection was incomplete.

Relationship between the pharmacokinetics of nicardipine and renal function

There was a significant inverse correlation between the AUC of nicardipine during chronic treatment and GFR ($r = -0.73$, $p = 0.005$, $n = 13$) and ERPF ($r = -0.68$, $p = 0.01$, $n = 13$) (figure 7.6.). When the magnitude of change in AUC during the translation from acute to chronic dosing was correlated with renal function this also showed significant correlations between ΔAUC and GFR ($r = -0.59$, $p = 0.03$, $n = 13$) or ΔAUC and ERPF ($r = -0.55$, $p = 0.05$, $n = 13$) (figure 7.7.).

Blood pressure and heart rate

The blood pressure and heart rate after acute and chronic dosing with nicardipine are shown in table 7.9. In relation to the baseline blood pressure, supine blood pressure was reduced after dosing to a lowest value at about 2 hours. In hypertensive patients with normal renal function (group1) blood pressure was reduced after chronic oral dosing from 162/98 $\pm$ 21/12 mmHg before the dose to 141/88 $\pm$ 10/6 mmHg 2 hours after the dose ($P < 0.03$ for the reduction in systolic blood pressure). In patients with renal impairment (group2), supine blood pressure was 169/97 $\pm$ 22/9 mmHg before dosing and 147/84 $\pm$ 25/11 mmHg 2 hours after dosing (statistical significance achieved for both systolic and diastolic blood pressure; $P < 0.04$). Supine heart rate was not affected by chronic nicardipine although there was an upward trends at 3 hours after dosing.

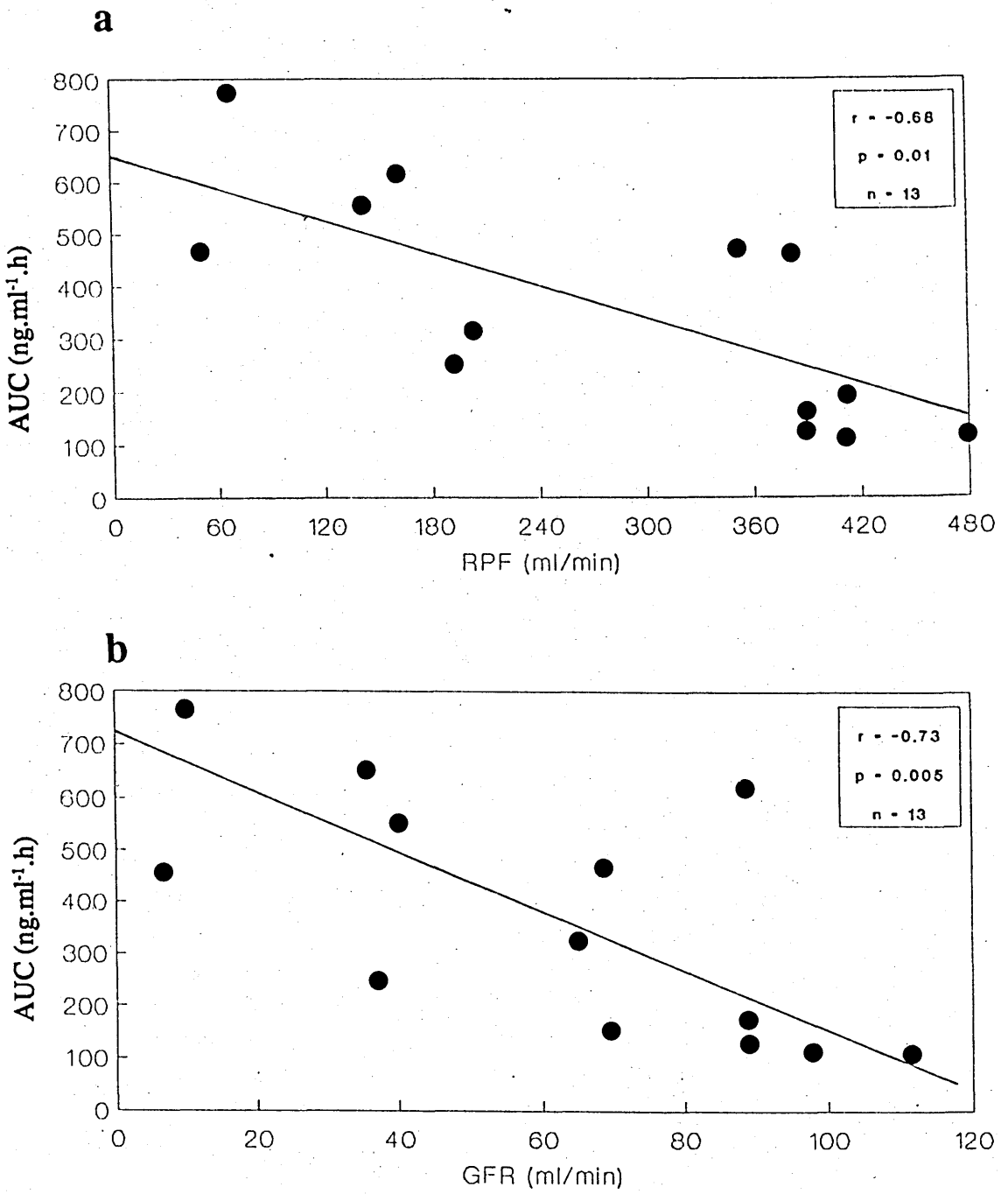


Figure 7.7. Relationship between AUC with chronic dosing of nicardipine and renal function; RPF (a), GFR (b).

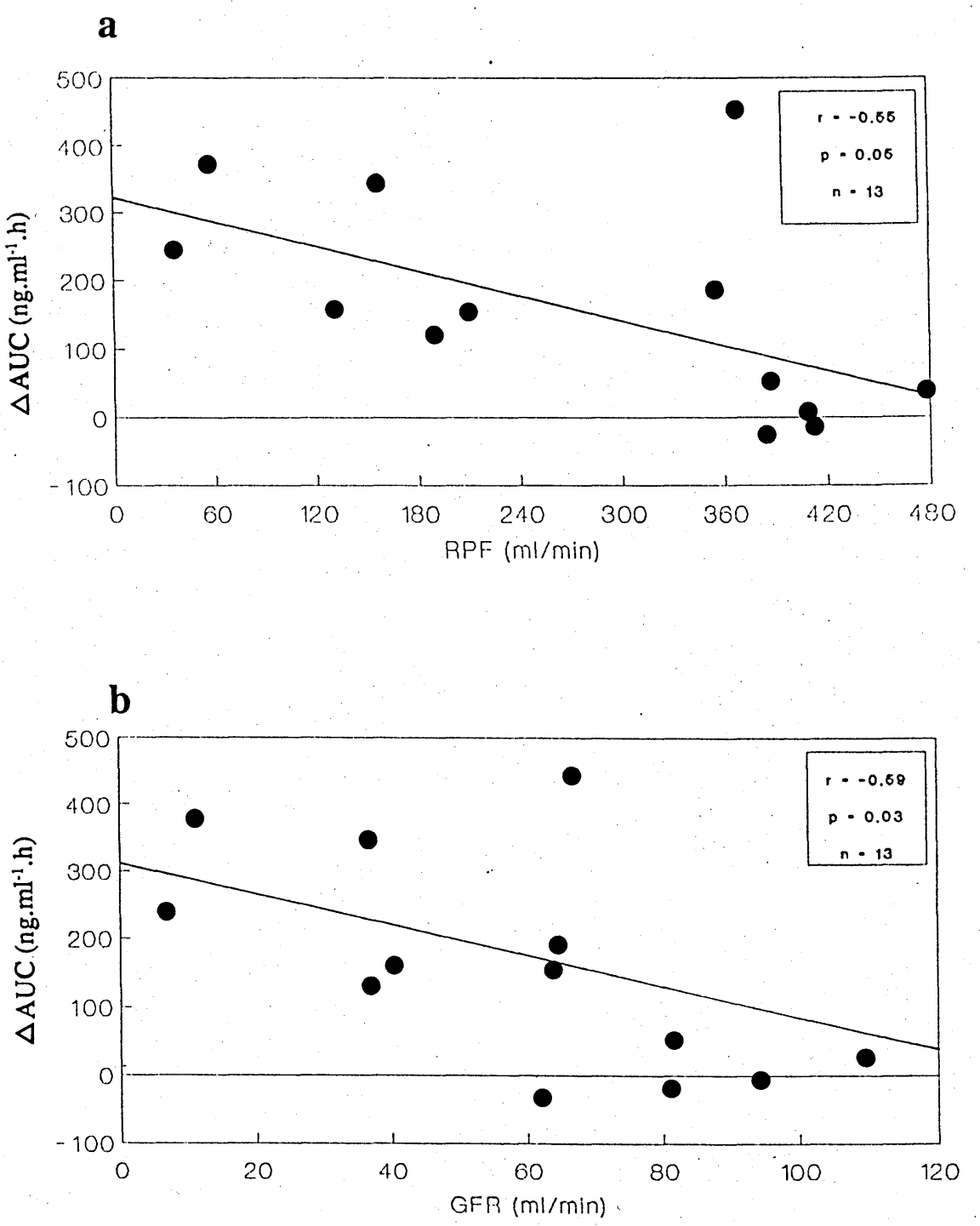


Figure 7.8. Relationship between the magnitude of change in AUC from acute to chronic dosing with nicardipine and renal function; RPF (a), GFR (b).

TABLE 7.9.

SUMMARY OF BLOOD PRESSURE AND HEART RATE AFTER TREATMENT WITH NICARDIPINE IN PATIENTS WITH DIFFERENT DEGREES OF RENAL IMPAIRMENT

Group	**Pre-dosing**		**2-hours post dose**	
	BP	HR	BP	HR
Group 1				
Acute I.V.	164/100±19/12	72±4	147/90±12/8	69±6
Acute oral	170/100±27/11	71±9	*146/87± 6/9	70±9
Chronic oral	162/98 ±21/12	70±9	*141/88±10/6	68±8
Group 2				
Acute I.V.	173/100±45/14	80±13	158/91±35/16	70±9
Acute oral	174/104±41/20	81±13	153/86±33/13	80±16
Chronic oral	169/97 ±22/9	81±7	*147/*84±25/11	80±14
Group 3				
Acute I.V.	147/82 ±17/11	75±6	144/80±17/13	69±6
Acute oral	152/83 ±21/14	76±4	147/79±13/11	74±7

* Significantly different from pre-dose value, $P < 0.05$; (ANOVA).

DISCUSSION

The positive findings, by many investigators, of the relationship between liver disease and reduction in the plasma clearance of calcium antagonist drugs and the absence of a correlation between renal impairment and altered pharmacokinetics for example with verapamil (Mooy et al., 1986), nifedipine (Kleinbloesem et al., 1985), diltiazem (Pozet et al., 1983) and nitrendipine (Bortel et al., 1989), has supported the expected theoretical concept that the liver is the major site for the metabolism of these drugs. This has largely ignored the potential importance of renal disease on hepatic metabolic capacity and the possible resultant impact on the metabolism of other dihydropyridine calcium antagonists. This has led to a general assumption that renal disease has no effect on the pharmacokinetics of calcium antagonists (Chellingsworth and Kendall, 1987). The present study has shown that the plasma clearance of nicardipine was significantly reduced in patients with renal impairment but restored towards normal in patients with end stage renal failure undergoing haemodialysis. These results are in agreement with other previous studies which showed that the AUC after dosing with nicardipine (20 or 30 mg orally) was significantly higher in patients with renal impairment compared to normal subjects (Lee et al., 1986). Similar results have been recently reported for nitrendipine (Ankermann et al., 1989). In these studies the effect of haemodialysis on drug disposition was not studied. There are only a few studies of calcium antagonist pharmacokinetics in

patients with end stage renal failure on regular haemodialysis and for nifedipine there were no differences (Martre et al., 1985; Kleinbloesem et al., 1986).

The mechanisms underlying the reduction in clearance in renal disease are not well defined but presumably reflect functional rather than structural changes. The overall correlation between the pharmacokinetic parameters, such as AUC, and the level of renal function suggests that the reduction in plasma clearance of nicardipine is related either directly to the deterioration in renal function (which is unlikely) or indirectly to the effects of renal dysfunction on hepatic metabolism. After oral administration nicardipine is subject to extensive hepatic metabolism (Higuchi et al., 1977; Graham et al., 1985) which may be saturable at relatively low concentrations (Seki and Takenaka, 1977; Silke et al., 1984). Thus the clearance of nicardipine ultimately depends on both liver blood flow and hepatocellular function. However, in this study there were no differences between the groups for apparent liver blood flow. Alternatively, the positive and significant correlation between the level of renal function and the magnitude of change in AUC, during the translation from acute to chronic dosing, might suggest that the reserve of the capacity limited hepatic enzyme system was reduced or inhibited in patients with renal impairment, so that hepatic metabolism was more easily saturated. Another explanation for the reduction in clearance is accumulation of renally-eliminated metabolites (Pyridine II) which in turn saturate or inhibit

or inhibit further metabolism. Although data on the metabolites were not available, it can be presumed that the generation of metabolites is less rapid and less marked after intravenous dosing and yet the plasma clearance after intravenous administration was still reduced in renal impairment.

Renal disease is frequently associated with alterations in protein binding resulting in increases in free drug concentrations which can more readily be distributed into tissues with a subsequent increase in the volume of distribution. The protein binding of two dihydropyridine derivatives nifedipine and nitrendipine (Kleinbloesem et al., 1985; Ankermann et al., 1989) was found to be reduced in patients with renal impairment resulting in a significant increase in volume of distribution. The protein binding in the present study was about 98% for the three groups and the volume of distribution was not different between groups 1 & 2.

It is perhaps surprising that patients with end stage renal failure on regular haemodialysis behave similarly to hypertensive patients with normal renal function. It seems that the haemodialysis treatment, which occurred on the days before the pharmacokinetic study days, was able to restore metabolic activity towards normal, possibly, by clearing the blood of an inhibitory substance which interfered with hepatic metabolism.

Increased plasma levels of nicardipine in renal impairment might potentially lead to enhanced effect.

Exaggerated responses to nifedipine (reduction in diastolic blood pressure) have been reported in renal impairment but these apparently were unrelated to plasma drug levels (Kleinbloesem et al., 1985). No such enhanced effects on blood pressure or heart rate are confirmed in the present study.

This study emphasises the importance of studying the pharmacokinetics of drugs which are highly extracted by the liver in patients with renal impairment even if the kidney is not the major site of elimination.

CHAPTER EIGHT

EFFECTS OF NICARDIPINE ON THE METABOLIC RESPONSES TO FOOD AND EXERCISE

INTRODUCTION

The failure of conventional antihypertensive drug regimens, based upon thiazide diuretics and beta blockers, to produce a significant impact on the incidence of coronary heart disease has raised the possibility that adverse metabolic effects, and particularly changes in lipids and lipoproteins, might be compromising the beneficial effects of blood pressure reduction. The conventional approach to the assessment of metabolic effects during long-term antihypertensive treatment is to monitor the relevant indices under basal, non-stimulated conditions before and after a period of drug treatment. An alternative approach, as described in this chapter, is to obtain hormonal and metabolic measurements in response to specific provocative stimuli (food and exercise). By stimulating metabolic and hormonal responses it may be possible to reveal a drug-related effect which might otherwise have been less readily detected under basal conditions.

Nicardipine is a dihydropyridine calcium antagonist for the treatment of hypertension and angina. Conventional studies of its effect on plasma lipids have elicited "neutral" results i.e. neither beneficial nor adverse effects (Trost & Weidmann, 1987) and similarly, studies in animals have shown no other significant adverse metabolic effects (Naito et al., 1984). This study has been undertaken to evaluate the effect of nicardipine on the metabolic and hormonal indices in response to standardised food and exercise challenge in patients with mild to moderate

essential hypertension.

SUBJECTS AND METHODS

Outline of study

Twelve patients (age 31-65 years; weight 72.5 ± 13 kg) with mild to moderate essential hypertension (supine diastolic blood pressure 90-110 mmHg) were recruited for a placebo controlled, double blind, randomised, crossover study which was approved by the Research and Ethical Committee of the northern District of the Greater Glasgow Health Board. Only 7 patients completed the study and their clinical characteristics are presented in table 8.1. The patients were randomly allocated to receive nicardipine 30 mg three times daily, or corresponding placebo tablets for a 4 weeks treatment period, at the end of which they attended the Clinical Pharmacology Research Unit for the first of 2 study days. Following a washout period of 7 days, patients then commenced a 4 weeks treatment period with the opposite treatment and duly attended for a second study day.

Study days

Having fasted overnight, patients attended the C.P.R.U. where the metabolic and hormonal responses to standard food and exercise challenges were evaluated on each study day whose design is shown in figure 8.1. Following the insertion of an indwelling intravenous cannula and a period of rest for 20 minutes, blood samples were collected for basal values of lipids, lipoproteins, glucose, insulin, thyroid

TABLE 8.1.

CLINICAL CHARACTERISTIC OF PATIENTS
WHO COMPLETED THE STUDY

Patients No.	Age Yrs	Weight Kg	Starting BP. mmHg	HR. bpm
1	63	78	164/101	76
2	58	72	171/100	87
3	64	72	173/99	80
4	60	64	169/104	74
5	55	86	190/109	81
6	59	53	172/104	80
7	61	85	150/93	72
Mean	60	72.4		
	3	12.6		

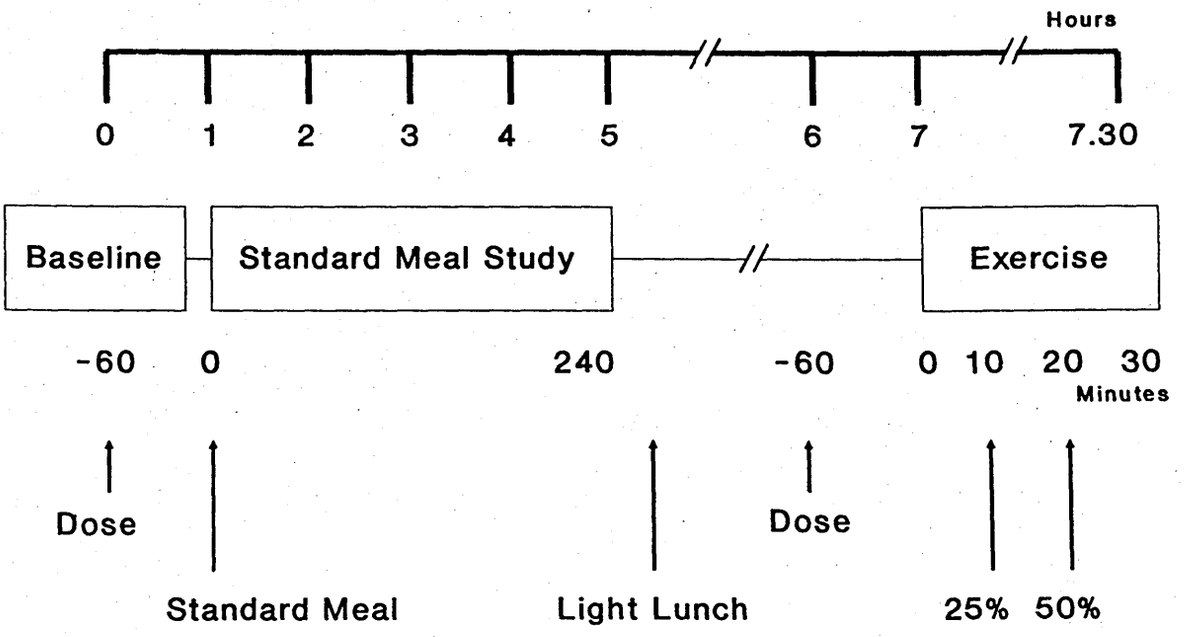


Figure 8.1. Schedule of study design.

stimulating hormones (TSH), renin, aldosterone and catecholamines. Blood pressure and heart rate were recorded in duplicate both supine and after 2 minutes in the erect position using a semi automatic sphygmomanometer (Sentron, Bard Medical). The morning dose of drug (or placebo) was then administered.

Responses to a standard meal

One hour after drug dosing, patients ate a standard meal containing 64 gm of carbohydrate, 25 gm of fat and 10 gm of protein with a total energy content of 2.3 m.j. (of which 40% was fat). The meal was prepared by the hospital dietetics department. Blood samples were collected before the meal and at 15, 30, 60, 120, 180, and 240 minutes thereafter for total cholesterol and cholesterol fractions, triglyceride, insulin and glucose.

Response to standard exercise

The maximal exercise capacity was predetermined according to the method described by Astrand and Astrand, 1958 using bicycle ergometery immediately after the patients had completed the standard meal study. Radial pulse was counted at the last 15 seconds of the 5th and 6th minutes, when the heart rate was stabilised, with subjects cycling against a resistance of 50-75 Watts for 6 minutes. The maximum oxygen uptake was calculated using a nomogram (figure 8.2.) and the maximum exercise capacity was calculated in "Watts" from a standard table (table 8.2.).

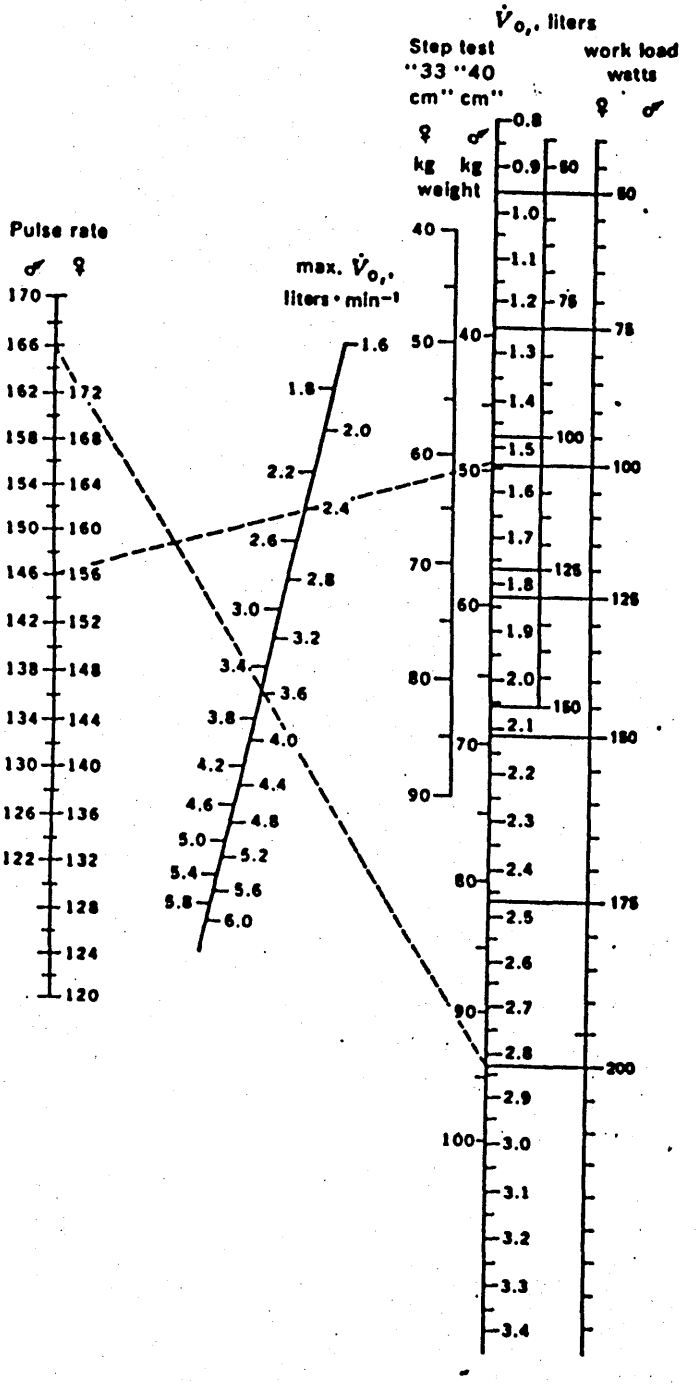


Figure 8.2. The adjusted nomogram for calculation of maximal oxygen uptake from submaximal pulse rate and oxygen uptake values. In test without direct oxygen measurements, it can estimated by reading horizontally from the "work load" scale (cycle test) to the oxygen uptake scale. A male subject reaches a heart rate of 166 bpm at cycling test on a work load of 200 watts; predicted max. Vo_2 = 3.6 liters/min. (Modified from Astrand and Ryhming, 1954).

TABLE 8.2.

PREDICTION OF MAXIMAL WORK LOAD (WATTS) FROM OXYGEN UPTAKE ON A BICYCLE ERGOMETER.

Oxygen uptake liters/min	Maximal Work Load Watts
0.9	50
1.5	100
2.1	150
2.8	200
3.5	250
4.2	300
5.0	350
5.7	400

A second dose of the assigned drug was then administered at 6 hours after the first (morning) dose and the standardised exercise test was then performed one hour after this second dose. The patients were exercised for two periods of 10 minutes at 25% and 50% of the individually predetermined maximal exercise capacity. Blood samples were collected before starting the exercise, at the end of the first load, at the end of the second load and after a rest for 10 minutes for the following: lipids, insulin, glucose, glucagon, growth hormone, cortisol, catecholamines, free fatty acid, renin, aldosterone and lactate. In addition, blood samples for the measurement of nicardipine levels were collected before the exercise and 10 minutes after the exercise had finished.

Plasma concentrations analyses

1) Serum lipids and lipoproteins were analysed by conventional enzymatic methods (Farish et al., 1986)
2) Free fatty acid was analysed by commercially available enzymatic kit (Wako Chemicals GmgH).
3) Glucose was measured by Beckman glucose analyser II (Glucose oxidase method).
4) Insulin, growth hormone, cortisol, renin, aldosterone, thyroxine and TSH were analysed by standard radio-immunoassays.
5) Immuno-reactive glucagon was analysed by courtesy of Professor K. Buchanan, Belfast.
6) Catecholamines were analysed by radio-enzymatic method (Peuler and Johnson, 1977).
7) Plasma lactate was analysed by enzymatic UV-method (Boehringer Manheim GmBH).

Calculation of AUC and maximal change

The area under the concentration time curve (AUC) was chosen to represent the response after the standard meal or exercise. It is calculated for baseline adjusted data by trapezoidal rule; for meal- from the start of meal to four hours after meal; for exercise- from the beginning of exercise to 30 minutes after exercise.

Maximal change was the peak achieved after the meal or exercise which was calculated directly from the graph.

For most variables maximal changes took place shortly after the meal or during the first or second load of

exercise but for some variables the maximum differences occurred later throughout the 4 hour study period. Accordingly, the data were analysed and presented both as maximal change after food or exercise and as baseline corrected profiles and AUC's.

Statistical analysis

Data throughout are presented for 7 patients as mean ± sd.

Time and treatment effects were evaluated by repeated measures analysis of variance using the statistical package "Rummage".

Paired Student's t-test was used to test differences in the base- line values or the maximal change after meal or exercise.

RESULTS

Responses to standard meal

The mean results for lipids and lipoproteins are presented in table 8.3. and 8.4. In response to the standard meal total cholesterol, cholesterol fractions, glucose and insulin increased to a peak at 15 to 30 minutes, and then declined towards the pre meal values. There were no significant differences between nicardipine and placebo, either in the peak response or in AUC of response, when allowance is made for the baseline differences.

Although the post meal profiles of both total cholesterol and HDL cholesterol were higher after nicardipine (figure 8.3. and 8.4.) by analysis of covariance (using the baseline values as covariate) there were no

significant differences between the two treatments. Blood glucose and serum insulin increased after the meal, both after placebo and after nicardipine, with peak values between 30 and 60 minutes and thereafter a decline towards pre meal value. Statistical analysis showed no differences between the two treatments (figure 8.5.).

Response to standardised exercise

The mean results for biochemical values before and during exercise are listed in table 8.5. and the response expressed as AUC's adjusted for baseline are listed in table 8.6. During the exercise test the levels of total cholesterol and of the cholesterol fractions increased and were slightly higher after nicardipine (figure 8.6. and 8.7.). For HDL cholesterol the maximal change was higher after nicardipine compared to placebo (0.04 $\pm$ 0.1 vs 0.1 $\pm$ 0.06 mmol/l) but statistical analysis showed no difference. There were no significant differences in the AUC between nicardipine and placebo.

Glucose, glucagon, and insulin were reduced during exercise (figure 8.8. and 8.9.) but statistical analysis could not detect differences between the two treatments. Serum cortisol was reduced during exercise but started to increase above pre exercise level when the patients rested after the exercise had finished. Plasma lactate was slightly and insignificantly increased during exercise. There were no significant differences between nicardipine and placebo for growth hormone, aldosterone, renin, adrenaline and noradrenaline.

TABLE 8.3.

MEAN RESULTS FOR LIPID AND LIPOPROTEIN FOLLOWING STANDARD MEAL

variable	Fasting level	0 mins	15 mins	30 mins	60 mins	120 mins	180 mins	240 mins
Total cholesterol (mmol/l)								
Placebo	5.95	5.88	6.04	5.81	5.71	5.80	5.80	5.98
sd	1.41	1.48	1.55	1.51	1.40	1.28	1.27	1.24
Nicardipine	6.05	6.12	6.45	6.18	5.89	5.92	5.90	5.98
sd	1.28	1.34	1.29	1.29	1.33	1.30	1.27	1.25
HDL cholesterol (mmol/l)								
Placebo	1.22	1.18	1.25	1.19	1.16	1.17	1.14	1.14
sd	0.29	0.28	0.29	0.28	0.27	0.28	0.28	0.24
Nicardipine	1.30	1.28	1.35	1.28	1.25	1.25	1.16	1.18
sd	0.24	0.22	0.23	0.22	0.23	0.25	0.21	0.21
HDL2 cholesterol (mmol/l)								
Placebo	0.34	0.36	0.38	0.40	0.37	0.38	0.35	0.31
sd	0.23	0.19	0.21	0.28	0.24	0.24	0.20	0.19
Nicardipine	0.33	0.35	0.35	0.30	0.32	0.35	0.34	0.33
sd	0.15	0.13	0.14	0.12	0.17	0.14	0.15	0.13
HDL3 cholesterol (mmol/l)								
Placebo	0.88	0.81	0.86	0.79	0.79	0.78	0.78	0.82
sd	0.12	0.12	0.13	0.13	0.07	0.09	0.12	0.12
Nicardipine	0.97	0.93	0.99	0.97	0.92	0.90	0.82	0.84
sd	0.23	0.26	0.26	0.24	0.22	0.18	0.18	0.16

TABLE 8.3. (Cont.)

variable	Fasting level	0 mins	15 mins	30 mins	60 mins	120 mins	180 mins	240 mins
LDL cholesterol (mmol/l)								
Placebo	3.92	4.04	4.18	4.04	3.94	3.97	3.95	4.04
sd	1.05	1.19	1.27	1.12	1.10	1.08	1.02	1.00
Nicardipine	4.01	4.07	4.37	4.15	4.00	3.90	3.90	3.84
sd	1.21	1.28	1.12	1.08	1.14	1.09	1.23	1.11
VLDL cholesterol (mmol/l)								
Placebo	0.65	0.65	0.60	0.61	0.60	0.64	0.70	0.80
sd	0.30	0.28	0.25	0.30	0.31	0.31	0.20	0.27
Nicardipine	0.74	0.77	0.75	0.72	0.72	0.78	0.84	0.97
sd	0.56	0.64	0.56	0.61	0.59	0.64	0.69	0.73
Triglycride (mmol/l)								
Placebo	1.81	1.72	1.82	1.77	1.94	2.25	2.48	3.08
sd	0.79	0.74	0.80	0.77	0.83	1.37	1.10	1.19
Nicardipine	2.17	2.22	2.37	2.32	2.34	2.47	2.95	3.38
sd	1.52	1.53	1.55	1.49	1.53	1.63	1.77	1.80
Serum insulin (MU/l)								
Placebo	12.38	11.78	27.85	65.28	98.57	78.00	37.71	20.62
sd	9.96	9.42	14.21	26.86	83.15	92.90	22.26	14.92
Nicardipine	13.48	14.15	30.28	58.71	100.71	52.31	30.85	19.15
sd	10.99	11.74	7.67	21.56	80.43	52.62	19.77	14.56
Blood Glucose (mmol/l)								
Placebo	5.40	5.51	6.27	8.41	8.01	6.07	5.31	4.78
sd	1.10	1.01	1.20	1.38	2.02	1.67	0.54	0.48
Nicardipine	5.42	5.72	6.70	8.92	7.90	6.10	5.24	5.12
sd	0.87	0.68	0.57	1.24	2.42	1.97	0.41	0.40

TABLE 8.4.

THE AREA UNDER THE CURVE ($AUC_{0-240\ MIN}$) (BASELINE CORRECTED) AFTER STANDARD MEAL FOR SERUM LIPIDS, INSULIN AND GLUCOSE.

	$AUC_{0-240\ min}$	
	Placebo	**Nicardipine**
1. Lipids		
(a) Total cholesterol (mmol/ml)	-14.2 ± 15	-30.2 ± 39
(b) HDL-C (mmol/l)	-4.7 ± 10	12.4 ± 10
(c) LDL-C (mmol/l)	-11.8 ± 50	-24.2 ± 64
(d) VLDL-C (mmol/l)	1.6 ± 22	8.0 ± 36
(e) Triglyceride (mmol/l)	130.0 ± 78	102.0 ± 33
2. Glucose (mmol/l)	189.0 ± 113	160.0 ± 176
3. Insulin (MU/l)	7234.0 ± 4360	8581.0 ± 5710

* The unit for AUC is concentration unit * min.

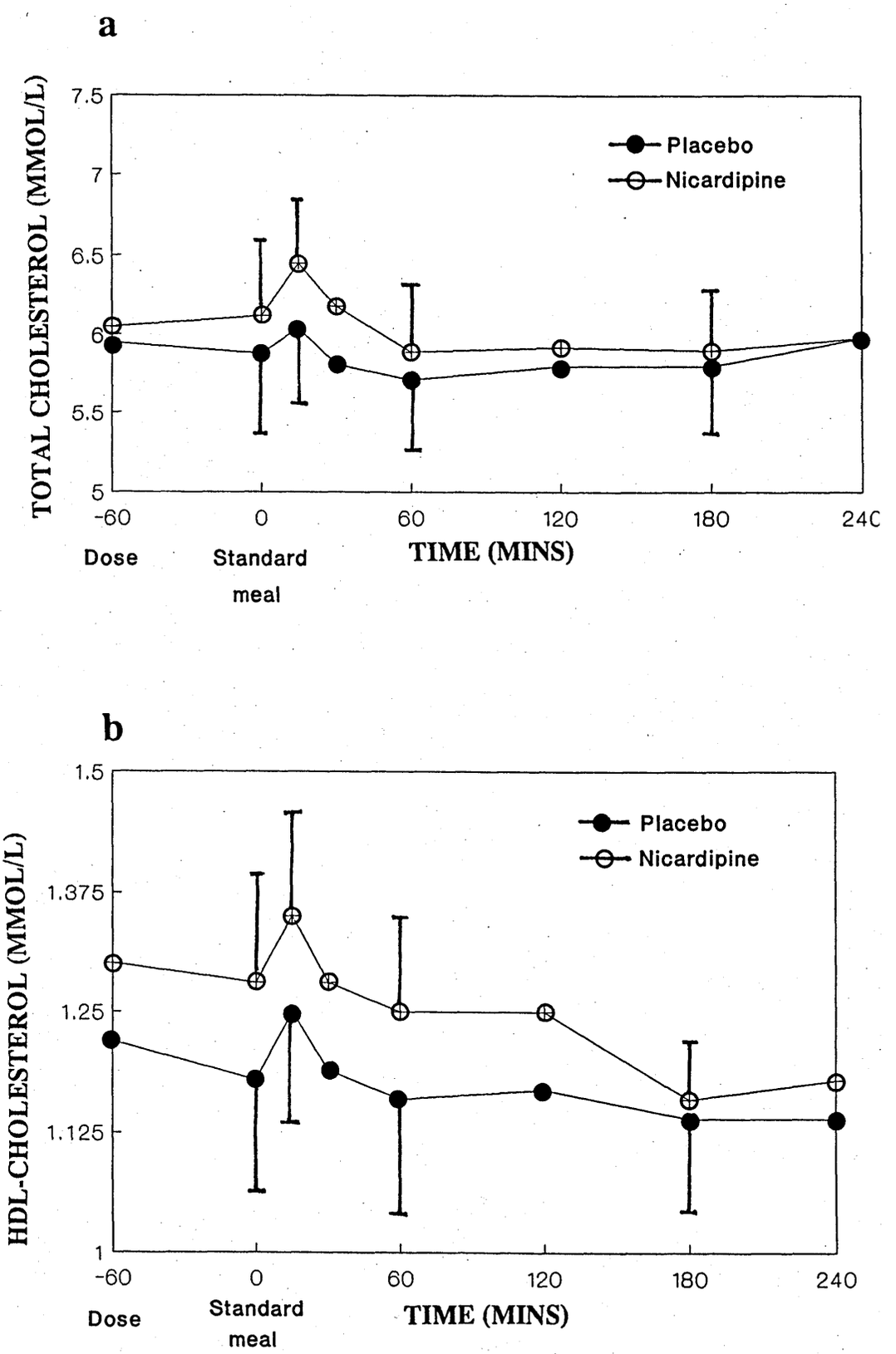


Figure 8.3. Total cholesterol (a) and high density lipoprotein cholesterol (b) responses to standard meal after placebo (●) or nicardipine (○). The data are presented as mean and error bars represents the standard error of the mean (SE).

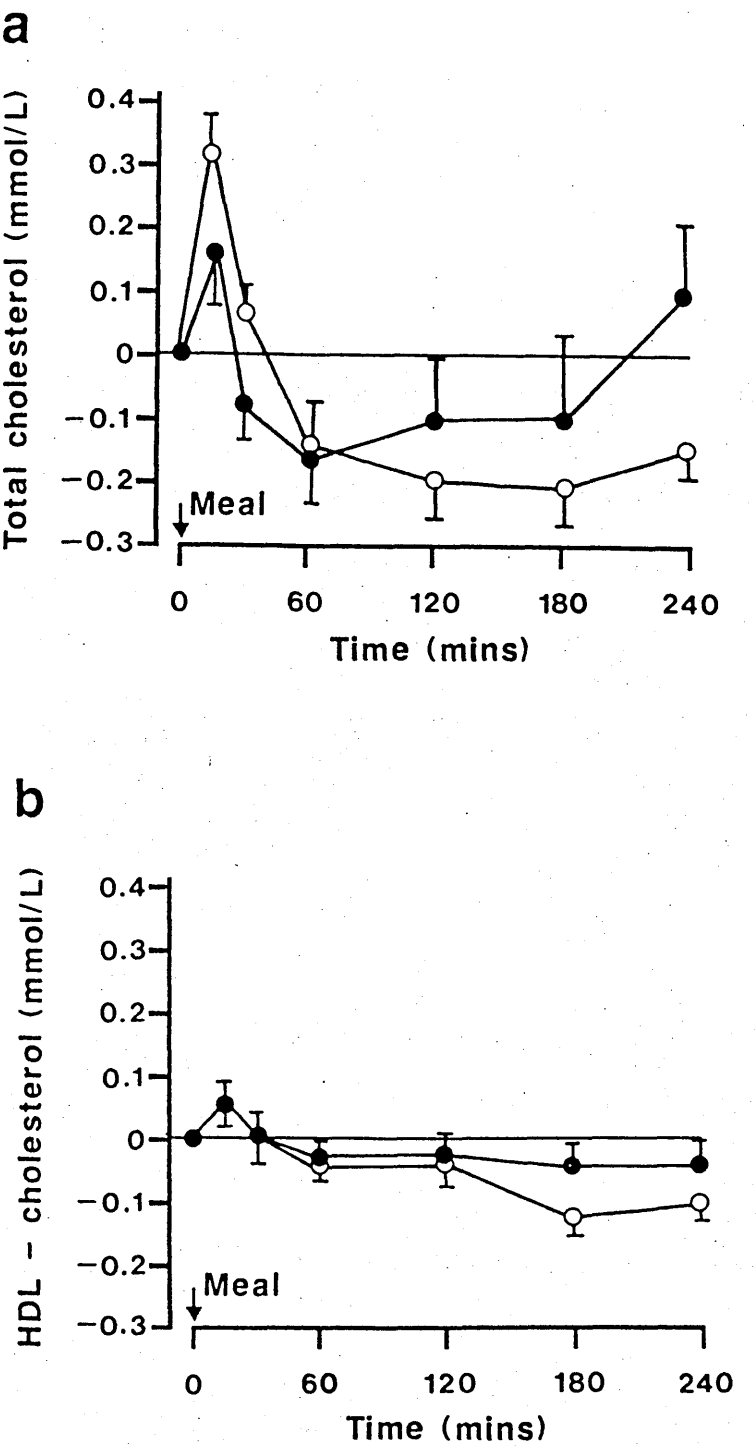


Figure 8.4. The change (baseline adjusted) for total cholesterol (a) and high density lipoprotein cholesterol (b) responses to standard meal after placebo (●) or nicardipine (O). The data are presented as mean and error bars represent the standard error of the mean (SE).

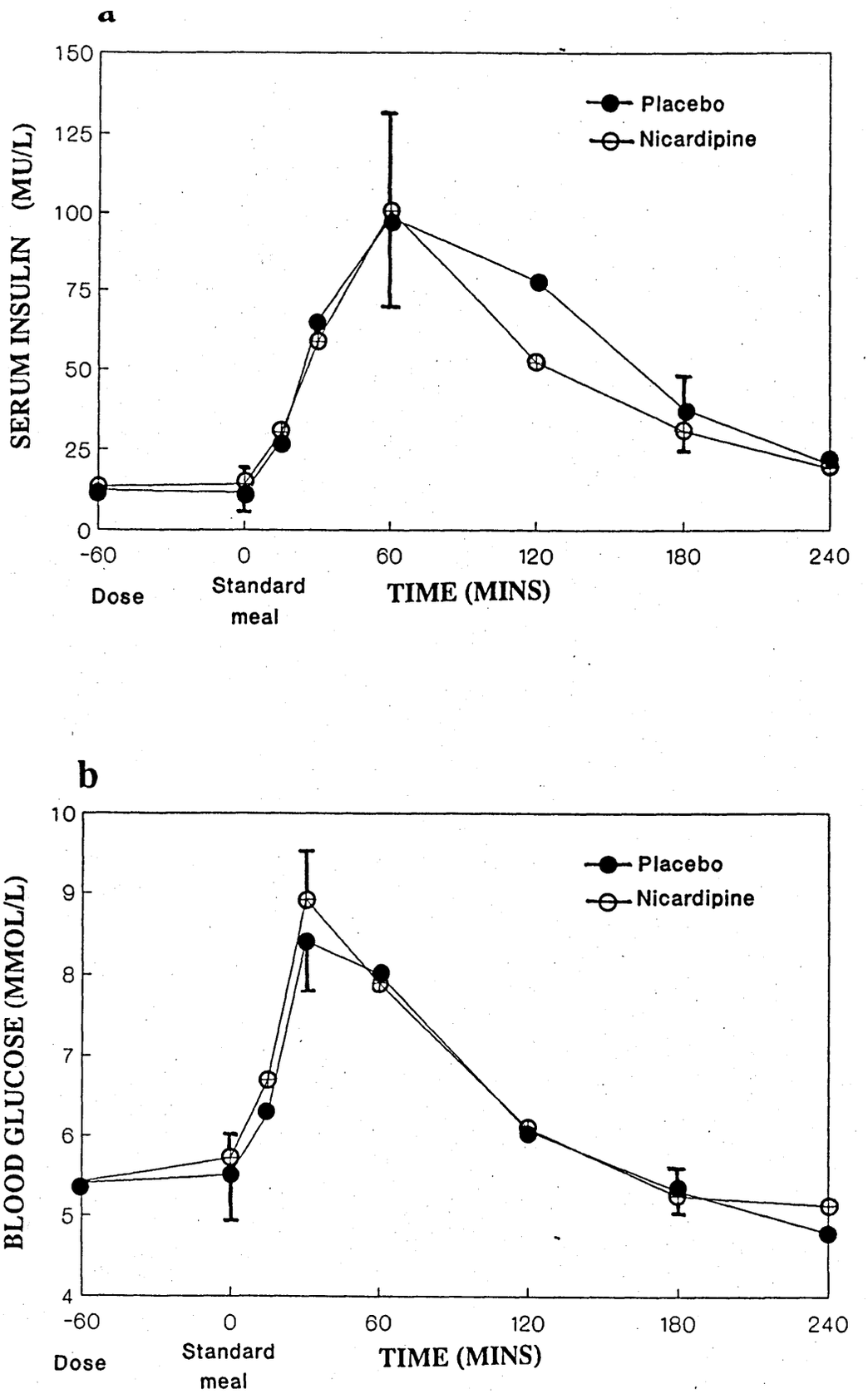


Figure 8.5. Serum insulin (a) and blood glucose (b) responses to standard meal after placebo (●) or nicardipine (○). The data are presented as mean and error bars represents the standard error of the mean (SE).

TABLE 8.5.

MEAN RESULTS FOR BIOCHEMICAL VALUES BEFORE EXERCISE AND DURING EXERCISE AND AFTER 10 MINUTES IN REST POSITION

Variable	Placebo				Nicardipine			
	Pre exercise	10 mins	20 mins	30 mins	Pre exercise	10 mins	20 mins	30 mins
Total cholesterol (mmol/l)								
Mean	6.02	6.37	6.44	6.22	6.10	6.35	6.48	6.36
sd	1.33	1.41	1.38	1.34	1.23	1.32	1.24	1.42
HDL cholesterol (mmol/l)								
Mean	1.09	1.14	1.13	1.13	1.11	1.18	1.21	1.20
sd	0.27	0.30	0.29	0.30	0.22	0.24	0.26	0.17
HDL2 cholesterol (mmol/l)								
Mean	0.33	0.30	0.20	0.26	0.32	0.25	0.24	0.28
sd	0.15	0.21	0.17	0.18	0.10	0.14	0.15	0.17
HDL3 cholesterol (mmol/l)								
Mean	0.76	0.83	0.94	0.87	0.79	0.93	0.97	0.91
sd	0.18	0.15	0.22	0.20	0.15	0.24	0.23	0.18
LDL cholesterol (mmol/l)								
Mean	3.91	4.07	4.31	4.15	3.90	4.11	4.27	4.20
sd	1.23	1.05	1.29	1.22	1.02	1.12	1.09	1.25
VLDL cholesterol (mmol/l)								
Mean	1.01	1.17	1.01	0.92	1.07	1.05	1.00	0.95
sd	0.50	0.46	0.44	0.52	0.47	0.42	0.49	0.51
Triglyceride (mmol/l)								
Mean	3.41	3.64	3.65	3.41	4.20	4.40	4.40	3.91
sd	1.60	1.60	1.66	1.61	2.09	2.24	2.27	2.43

TABLE 8.5. (Cont.)

Variable	Placebo				Nicardipine			
	Pre exercise	10 mins	20 mins	30 mins	Pre exercise	10 mins	20 mins	30 mins
Serum insulin (MU/l)								
Mean	56.50	39.00	38.75	49.40	55.40	44.51	29.01	41.28
sd	55.59	54.86	58.77	72.18	49.37	54.99	22.09	24.03
Blood glucose (mmol/l)								
Mean	5.51	5.15	5.05	5.20	5.25	4.91	4.41	5.41
sd	2.04	2.13	1.57	1.49	2.68	2.02	2.09	2.12
Glucagon (mmol/l)								
Mean	51.42	42.50	39.28	42.50	40.00	32.14	39.16	35.00
sd	40.48	11.72	18.35	26.59	15.00	6.98	4.91	8.66
Cortisol (nmol/l)								
Mean	219.85	177.66	236.33	321.85	285.42	270.00	268.57	397.50
sd	72.04	93.05	85.71	145.44	119.44	154.63	103.59	211.72
Growth hormone (MU/l)								
Mean	0.58	0.52	0.70	0.91	0.50	0.50	0.66	0.87
sd	0.22	0.07	0.41	0.57			0.26	0.62

TABLE 8.5. (Cont.)

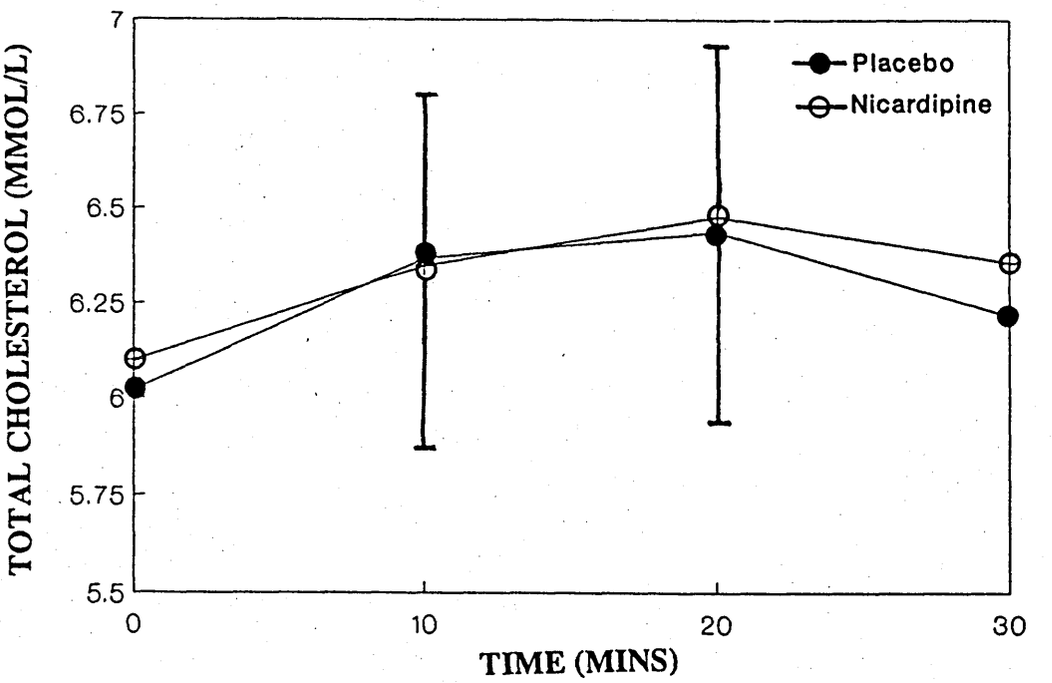
Variable	Placebo				Nicardipine			
	Pre exercise	10 mins	20 mins	30 mins	Pre exercise	10 mins	20 mins	30 mins
Free fatty acid (mmol/l)								
Mean	0.45	0.42	0.67	0.58	0.50	0.59	0.76	0.75
sd	0.10	0.15	0.58	0.25	0.21	0.24	0.27	0.25
Plasma lactate(mg/100 ml)								
Mean	19.73	24.55	31.37	24.53	17.16	21.30	34.32	26.48
sd	2.63	4.96	8.47	7.11	4.41	7.05	9.86	11.01
Plasma aldosterone (pg/ml)								
Mean	77.00	137.00	194.00	195.00	100.00	146.00	168.00	213.00
sd	40.00	80.00	96.00	65.00	59.00	38.00	55.00	65.00
Plasma renin (ng AI/ml/h)								
Mean	2.04	3.74	4.26	4.36	3.29	6.79	5.38	5.68
sd	1.05	3.64	3.45	3.25	1.54	5.85	2.54	3.13
Plasma adrenaline (nM)								
Mean	0.23	0.20	0.23	0.18	0.13	0.16	0.23	0.17
sd	0.21	0.07	0.07	0.04	0.02	0.05	0.17	0.07
Plasma noradrenaline (nM)								
Mean	1.17	1.31	1.09	1.01	1.53	1.59	1.79	1.45
sd	0.70	0.99	0.73	0.67	0.70	0.68	1.10	0.69

TABLE 8.6.

THE AREA UNDER THE CURVE ($AUC_{0-30\ MIN}$) (BASELINE CORRECTED) DURING EXERCISE FOR SERUM LIPIDS, LIPOPROTEINS AND HORMONES.

	*$AUC_{0-30\ min}$ Placebo	Nicardipine
1. Lipids		
(a) Total cholesterol (mmol/ml)	8.6 ± 4.0	7.4 ± 3.4
(b) HDL-C (mmol/l)	1.0 ± 1.8	1.9 ± 1.3
(c) LDL-C (mmol/l)	6.8 ± 2.8	6.8 ± 4.0
(d) VLDL-C (mmol/l)	1.1 ± 3.0	-1.1 ± 3.0
(e) Triglyceride (mmol/l)	5.0 ± 3.0	3.7 ± 7.0
2. Glucose (mmol/l)	-16.0 ± 20.0	-16.0 ± 27.0
3. Insulin (MU/l)	-388.0 ± 385.0	-443.0 ± 393.0
4. Glucagon (ng/ml)	-293.0 ± 925.0	-125.0 ± 237.0
5. Cortisol (nmol/l)	143.0 ± 708.0	22.8 ± 1317.0
6. Free fatty acid (mmol/l)	2.7 ± 6.0	4.7 ± 4.5
7. Lactate (mg/100 ml)	151.0 ± 104.0	259 ± 209

* The unit for AUC is concentration unit * min.



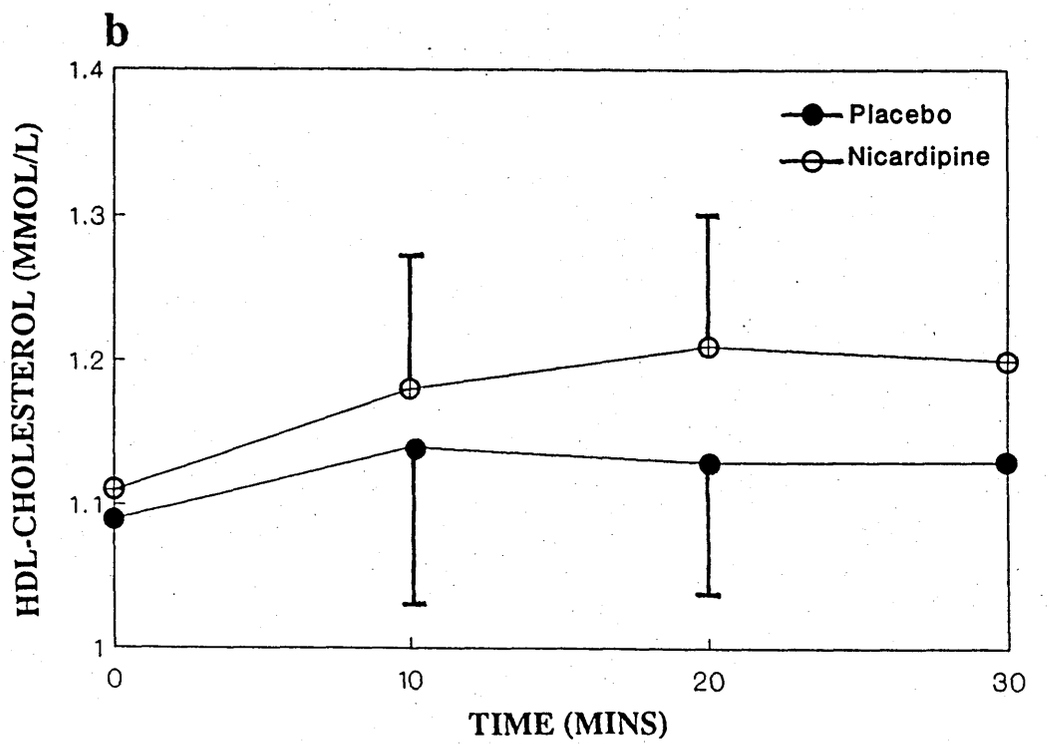


Figure 8.6. Total cholesterol (a) and high density lipoprotein cholesterol (b) responses to exercise after placebo (●) or nicardipine (O). The data are presented as mean and error bars represents the standard error of the mean (SE).

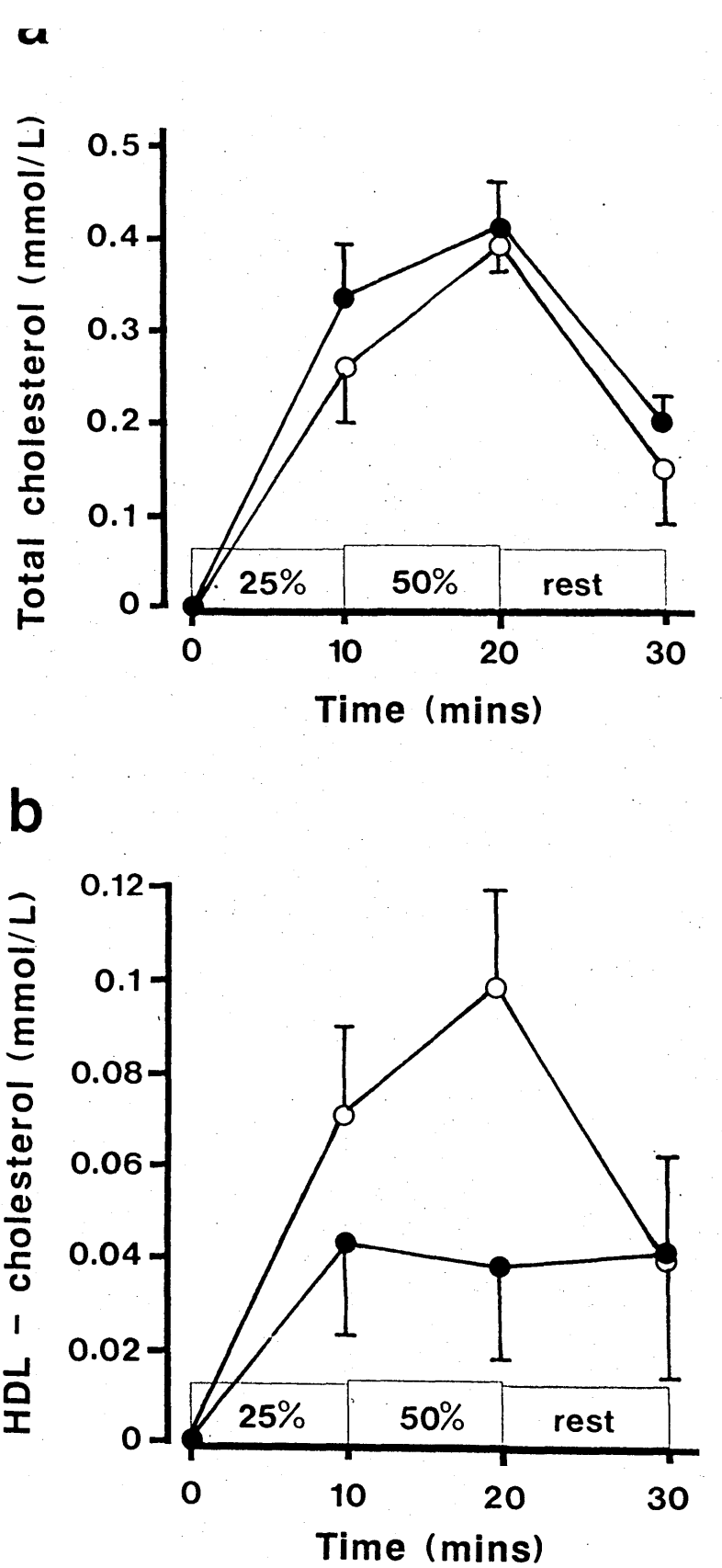


Figure 8.7. Response to exercise presented as the change (baseline adjusted) for total cholesterol (a) and high density lipoprotein cholesterol (b) after placebo (●) or nicardipine (O). The data are presented as mean and error bars represent the standard error of the mean (SE).

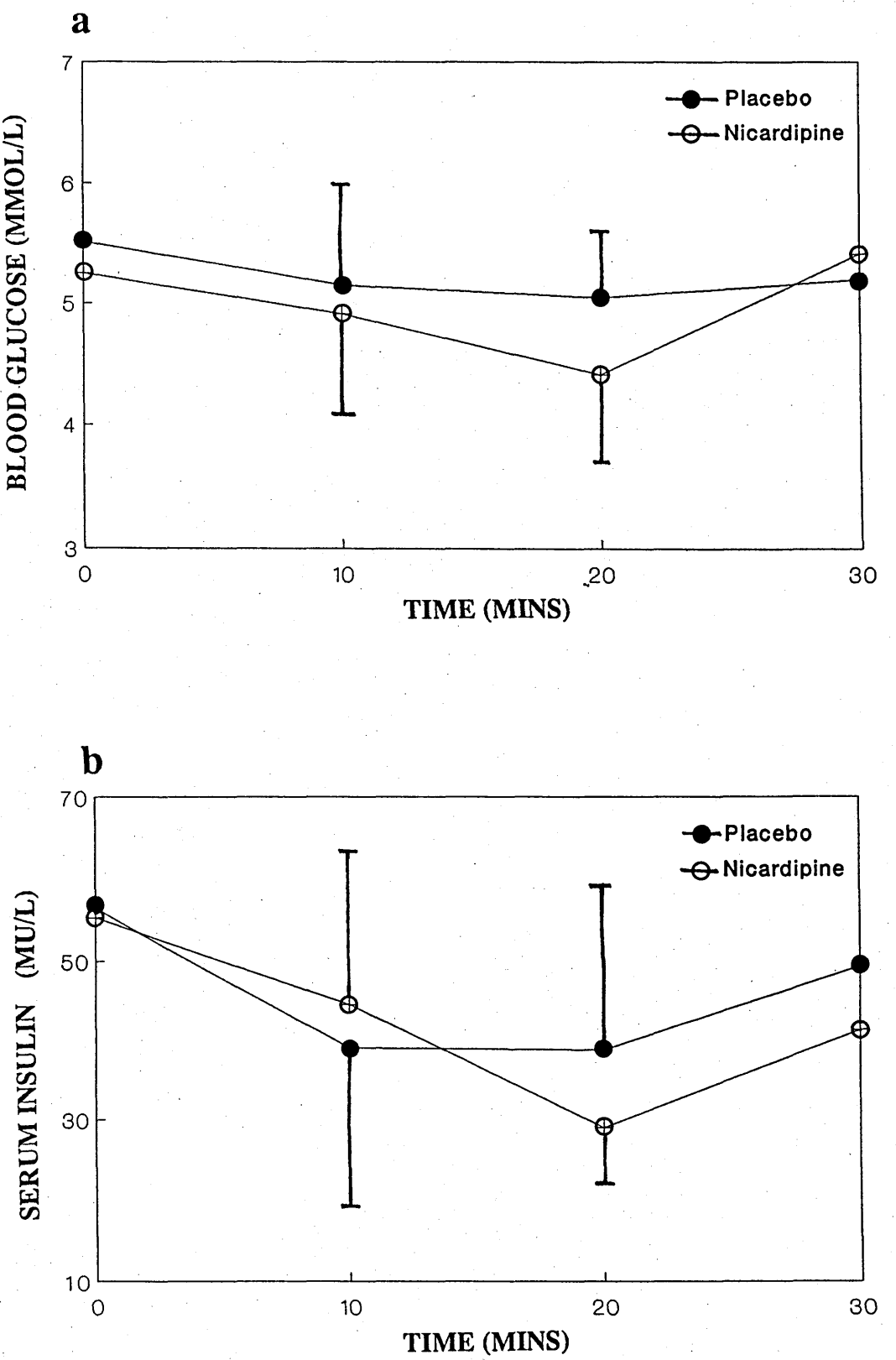


Figure 8.8. Glucose (a) and insulin (b) responses to exercise after placebo (●) or nicardipine (○). The data are presented as mean and error bars represents the standard error of the mean (SE).

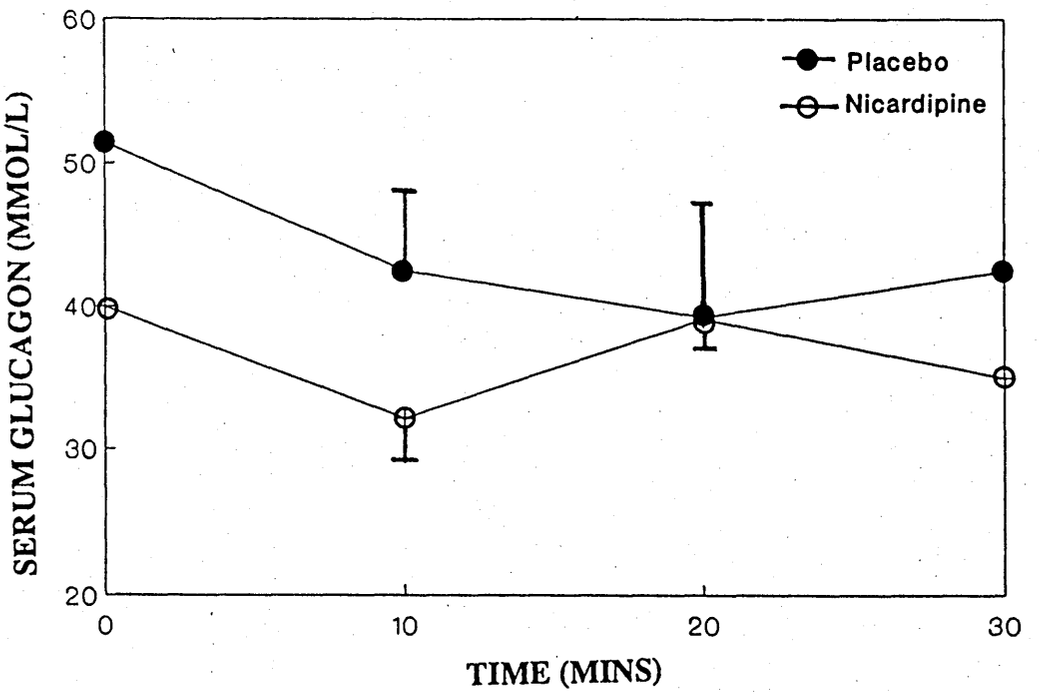


Figure 8.9. Glucagon response to exercise after placebo (●) or nicardipine (O). The data are presented as mean and error bars represents the standard error of the mean (SE).

Blood pressure and heart rate

Basal supine blood pressure was reduced after nicardipine compared to placebo (164/96 ± 31/10 vs 149/85 ± 16/10 mmHg) and statistical significance was observed for diastolic pressure. This was not associated with a change in heart rate (65 ± 9 vs 64 ± 8 bpm).

Basal levels (fasting:pre dosing)

There were small, variable and non-significant increases in all lipid fractions after nicardipine compared to placebo (table 8.3.). There were no significant changes in the fasting levels of serum insulin or blood glucose, nor in plasma adrenaline and noradrenaline. There was a statistically significant increase in the fasting level of plasma aldosterone, from 87 ± 56 pg/ml after placebo to 142 ± 60 pg/ml after nicardipine ($p < 0.01$) but plasma renin activity was not significantly increased by nicardipine (3.4 ± 2.5 vs 2.7 ± 1.6 ng AI/ml/hr on placebo). There was a small but significant increase in thyroid stimulating hormone (TSH) after nicardipine (1.6 ± 0.76 vs 1.16 ± 0.7 nmol/l; $p < 0.04$) although thyroxine was not affected (102 ± 11 vs 101 ± 13 nmol/l) (table 8.7.).

Adverse effects

Three patients were withdrawn from the study because of volunteered side effects: 2 during nicardipine treatment (One patient had fatigue, flushing, nausea and headache and the second had nausea) and 1 during placebo treatment (headache). Two further patients were found to have been

TABLE 8.7.

BASAL (FASTING) LEVELS OF RENIN, ALDOSTERONE, CATECHOLAMINE AND THYROID INDICES

Parameters	**Placebo**	**Nicardipine**
Aldosterone (pg/ml)	87 ± 56	142 ± 60 *
Renin (ng AI/ml/h)	3.3 ± 2.5	2.7 ± 1.4
Adrenaline (nM)	0.3 ± 0.2	0.3 ± 0.4
Noradrenaline (nM)	1.2 ± 0.7	1.2 ± 0.8
TSH (uIU/ml)	1.2 ± 0.7	1.6 ± 0.7 *
T4 (uIU/ml)	102 ± 11	101 ± 13

* Significant difference $P < 0.05$ between placebo and nicardipine.

inadequately compliant when tablet counts and plasma drug concentrations were measured and their results have been excluded. Thus, full metabolic and hormonal results were available for analysis on 7 patients. For the 7 patients who completed the study, mild and transient side effects were reported during the nicardipine phase: two patients had fatigue and one patient had headache after nicardipine.

DISCUSSION

Many metabolic functions, including the release of hormones, are partly dependent on the availability and intracellular influx of calcium ions and there is experimental evidence that hormone release can be blocked by calcium antagonists (Grodsky & Bennet, 1966; Devis et al., 1975). To complement the studies which have measured lipid profiles under basal circumstances this study was designed to investigate the effect of antihypertensive treatment with nicardipine on the metabolic and hormonal responses to two different physiological stimuli, feeding and exercise.

There was no significant effect attributable to nicardipine either under basal conditions or in response to food or exercise. Since complex metabolic and hormonal inter-relationships are involved in plasma lipid and lipoprotein regulation (Ganong, 1985) the interpretation of changes in serum lipid is complex. For example, lipoprotein lipase and hormone-sensitive lipase are two of the enzymes involved in lipid metabolism and there is evidence that they are differently affected by feeding and stress (Ganong,

1985). The activity of hormone-sensitive lipase is increased in the fasting state and decreased by feeding, and by insulin (Ganong, 1985). In addition, hormone sensitive lipase can be activated by increases in cyclic AMP in response to catecholamines, glucagon and TSH, and by a slower process by other hormones, such as growth hormone and cortisol (Ganong, 1985). In contrast, feeding increases the activity of lipoprotein lipase whereas fasting and stress decrease it. Similarly, changes in plasma volume, or in catecholamine levels, might indirectly influence metabolism but there was no evidence of any such changes in this study.

In addition, it has been proposed that abnormalities of glucose and insulin metabolism have a role in both the aetiology and the clinical course of hypertension and some antihypertensive drugs are associated with deterioration in glucose tolerance (Reaven et al., 1987). In this clinical study, normal insulin release and glucose-stimulated insulin release were not affected by nicardipine. This lack of significant interference with insulin release, which is in agreement with earlier results with nicardipine (Dow et al., 1985), is consistent with a concentration-dependent effect since there is evident from <u>in vitro</u> studies to show that the concentration needed to interfere with insulin release is much greater then the concentration known to reduce vascular tone (Al-mahmoud et al., 1986; Marre et al., 1986).

In the present study short-term treatment with nicardipine for 1 month was accompanied by significant increase in the level of aldosterone, while the change in

plasma renin activity was not significant. This finding is in agreement with other short-term and chronic studies with calcium antagonist drugs (Weber et al., 1987; Staessen et al., 1989).

The small increase in thyroid stimulating hormone (TSH) is surprising although previous studies (Isles et al., 1986) also have shown a small and insignificant increase in TSH with nicardipine. The mechanism behind the increase in TSH is not clear. However, there is some evidence that the acute intravenous infusion of calcium to normal subjects reduces prolactin and TSH release (Feely et al., 1986) and it is possible that the blockade of calcium influx will increase the release of TSH.

The impact of adverse drug-related metabolic effects is obviously a long-term problem and it might be argued that a 4-week treatment period is insufficient. However, since calcium ions are involved in acute processes such as hormone release, and not synthesis, it is likely that 4 weeks would be enough to show an effect. There is evidence in both patients and volunteers that treatment periods of only 2 weeks are sufficient to demonstrate effects on lipoprotein metabolism with the beta blockers atenolol, propranolol and pindolol (Durrington et al., 1985).

In conclusion this study of relatively short-term treatment with nicardipine has shown no evidence of any adverse-related metabolic or hormonal effect. The "provocative" approach used in this study, which is seen as complementary to long-term evaluations under basal

conditions, has confirmed the "lipid-neutral" status of the drug.

GENERAL DISCUSSION

GENERAL DISCUSSION

The principal aim of this thesis was to investigate aspects of the clinical pharmacology of calcium antagonist drugs, particularly in relation to issues arising in clinical practice such as occur in elderly patients with hypertension and angina and with special attention to renal function and metabolic effects.

Since the proportion of individuals aged 65 years and older is increasing in Western society there is an obvious and increasing need to direct attention to the pharmacokinetic and pharmacodynamic characteristics of drugs which are commonly used in elderly patients.

There is now increasing evidence that the pharmacokinetics of many calcium antagonist drugs are altered in elderly patients so that the plasma clearance may be reduced and elimination half life increased. Such changes have been reported with nifedipine (Robertson et al., 1988), amlodipine (Elliott et al., 1988), nitrendipine (Kendall et al., 1987; Crome et al., 1987; Van Harten et al., 1989) and with verapamil (Abernethy et al., 1986). Our findings with verapamil (chapter 5) confirmed that age-related changes in pharmacokinetics led to small but significant increases in peak plasma levels probably as a reflection of a reduction in drug clearance. However, it was also noted that the variability in the pharmacokinetic parameters increased markedly with age. Overall, it was concluded that dosage adjustment based solely on patient's age is inappropriate. Furthermore, to consider dosage adjustment solely on the

basis of pharmacokinetic differences requires that a clear relationship exists between concentration and effect. There is now evidence of a direct, although not simple, relationship between the plasma levels of verapamil and its antihypertensive effects (chapter 3). The "effect modelling" method of characterising responsiveness by integrating the kinetic and dynamic information (i.e. fall in blood pressure (mmHg) per unit change in drug concentration) additionally can be used to identify factors, such as age, which might influence the response.

Buhler and colleagues (1982) were the first to suggest that the antihypertensive effect of calcium antagonist drugs was greater in the elderly. This was attributed to increased tissue responsiveness, whether direct or indirect, and did not take account of any age related alteration in pharmacokinetics. If the elderly were truly more responsive to calcium antagonists then not only would there be advantageous increases in therapeutic responses but more critically, perhaps, there might be an increased risk of adverse effects, particularly on myocardial contractility or A-V conduction. The design of the study of single and multiple doses of verapamil in patients with angina pectoris, (chapter 4) on the basis of reports of age-related alterations to the pharmacology of verapamil and also the possibility of increased risk of adverse cardiac effects, therefore employed a relatively low range of doses. No such increase in adverse effects was observed and there was no significant effect on left ventricular ejection fraction,

either at rest or during exercise, and there was no significant effect on cardiac conduction (PR interval). The conclusion of this study was that the elderly did not show exaggerated responses to verapamil in terms of adverse effects on cardiac function. The lack of effect on PR interval in this study which is in broad agreement with a previous report by Abernethy et al (1986) suggests that the sensitivity to verapamil-induced prolongation in electrographic PR interval is less in the elderly than the young.

The knowledge of the antihypertensive effects of calcium antagonists is increasing rapidly but of equal importance is information about their effects on end-organ blood flow, particularly on the kidneys, since hypertension and renal disease frequently coincide. The effects of calcium antagonists on renal function were extensively reviewed recently (Chellingsworth and Kendall, 1987; Schlueter and Batlle, 1989) and in this thesis has been evaluated with verapamil (chapter 3 and 4) and with nicardipine (chapter 7). The results of these studies showed that acute and chronic dosing with these drugs had no deleterious effect on glomerular filtration rate or renal plasma flow. This result, which is confirmatory to other studies (Lee et al., 1986; Littler et al., 1986; Baba et al., 1987; Cox et al., 1988), has emphasised the clinical usefulness of calcium antagonists in the treatment of hypertension without reducing (and in some reports, improving) renal function. Whether or not calcium antagonist

drugs actually improve renal function remains controversial. To optimise dosage requirements, it is important to identify whether or not renal disease has any effect on the pharmacokinetics of calcium antagonist drugs. Our results (chapter 7) demonstrated that renal impairment reduced the plasma clearance of nicardipine resulting in increases in plasma levels. Although, we were not able to demonstrate an enhanced effect on blood pressure in this group of patients with impaired renal function, other workers have shown that the antihypertensive effects of the calcium antagonist, nifedipine, may be increased in patients with renal impairment (Kleinbloesem et al, 1985). Therefore, despite the prior assumption that no dosage adjustment would be required in hypertensive patients with renal impairment since nicardipine was not renally eliminated, this study showed that there was altered drug metabolism, leading to higher plasma drug concentrations and potentially to increased drug response.

Since elevated blood pressure is a risk factor for the development of coronary heart disease (CHD) and since there are also positive correlations between CHD and plasma levels of cholesterol and low density lipoprotein (LDL) cholesterol (and a negative correlation with high density (HDL) cholesterol) (Castelli et al., 1977; Miller et al., 1977; Gordon et al., 1977; Hulley et al., 1980; Yarri et al., 1981; Castelli et al., 1986) there has been recent concern that antihypertensive drugs should not worsen the plasma lipid profiles. Recent reviews of the metabolic and hormonal

effects of different antihypertensive drugs (Ames, 1986a; Ames, 1986b; Trost and Weidmann, 1987) have depended upon "longitudinal" studies i.e. with measurements made under basal conditions before and after treatment, for variable time periods, with the antihypertensive drugs. Many such studies have failed to incorporate adequate placebo data. The metabolic and hormonal effects of nicardipine have been evaluated, using a "provocative" approach, and confirmed that it has a "neutral" effect on plasma lipids, lipoproteins and hormones (chapter 8). The relevance of drug-induced lipid changes remains to be established but more accurate assessment of the different drug effects would be possible if a standardised "test" could be applied.

Most of the effects of calcium antagonist drugs described in this thesis can be explained on the basis of calcium entry blockade. However, it is not surprising with this heterogeneous group of drugs that they have other effects which cannot fit simply into the concept of their calcium entry blockade activity. Such an example is the inhibition of the metabolism of drugs which are metabolised by the liver (Pochet et al., 1986; Batch et al., 1986; Hunt et al., 1989). Another potentially more clinically applicable use is in the treatment of cancer. Although, many calcium antagonist drugs (verapamil, diltiazem and several dihydropyridines) (Tsuruo et al., 1983b see Endicott and Ling, 1989 for review) have been tested <u>in vitro</u> and have shown activity as modulators in resistant malignant cells, verapamil has been shown to be the most potent agent but in

relatively high concentrations (6 uM; 2700 ng/ml). Such concentrations may be more readily achieved in vivo in man, without cardiovascular toxicity, using the d-isomer verapamil. From the nature of drug resistance there is no one general modulator which can be used in all type of resistant cells (Tsuruo et al., 1983a) thus the field of cancer research is in need of the introduction of more modulators to provide the oncologists with a flexible range of choice.

In conclusion, calcium antagonist drugs are likely to make an increasingly important contribution to the effective treatment of cardiovascular (and other) disorders. They have no significant adverse impact on end-organ blood flow; they have a neutral effect on serum lipid and hormone profiles; they have no serious age-related toxicity; and, potentially, their use may be extended to the field of cancer research as adjuvant agents to overcome drug resistance.

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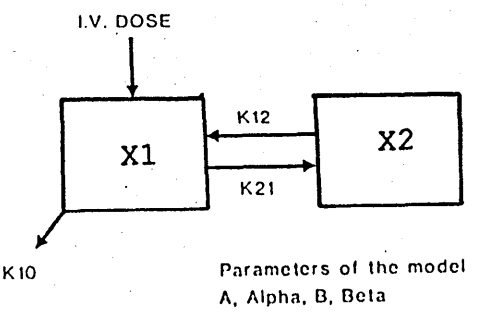
APPENDIX 1

Derivation of the kinetic model

1. **Intravenous Bolus Administration:**



$$dX1/dt = -(K12 + K10).X1 + K21.X2 \quad \text{——— 1}$$

$$dX2/dt = K12.X1 - K21.X2 \quad \text{——— 2}$$

From equation 1

$$S.L(X1) - X1(0) = -(K12 + K10).L(X1) + K12.L(X2) \quad \text{——— 3}$$

From equation 2

$$S.L(X2) - X2(0) = K12.L(X1) - K21.L(X2) \quad \text{——— 4}$$

Since this is the first dose

then: $X1(0) = D$, $X2(0) = 0$

Thus equation 4 can be simplified to:

$$L(X2) = \frac{K12.L(X1)}{(S + K21)} \quad \text{——— 5}$$

Substituting in equation 3

$$S.L(X1) - D = -(K12 = K10).L(X1) + \frac{K12.K21.L(X1)}{(S + K21)}$$

$$D = \frac{S^2 + (K12 + K10 + K21).S + (K10.K21).L(X1)}{(S + K21)}$$

$$\text{i.e. } L(X1) = \frac{D.(S + K21)}{S^2 + S.(K12 + K10 + K21) + K21.K10}$$

$$\text{i.e. } L(X1) = \frac{D.(S + K21)}{(S + \alpha)(S + \beta)}$$

Where $\alpha\ \beta = K21.K10$

$\alpha + \beta = K10 + K12 + K21$

L(X1) is now in a standard form so that from the table of inverse Laplace Transforms (table A1, function 9.),

$$X1 = \frac{D}{(\alpha - \beta)} (\alpha - K21).e^{-\alpha t} - (\beta - K21).e^{-\beta t})$$

and

$$C(t) = A.e^{-\alpha t} + B.e^{-\beta t} \qquad \text{6}$$

Where $A = \frac{D.(\alpha - K21)}{V1(\alpha - \beta)}$, and $B = \frac{D.(K21 - \beta)}{V1(\alpha - \beta)}$

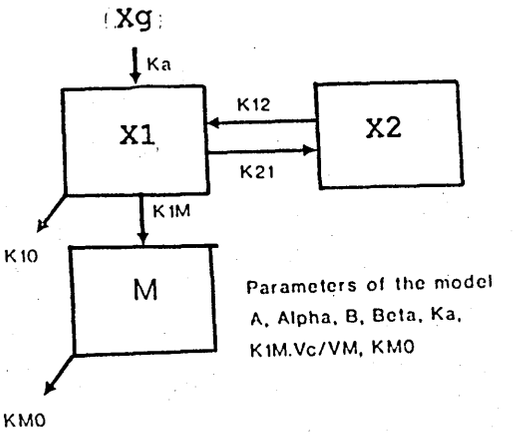
V1 = Volume of the central compartment.

TABLE A1

LAPLACE TRANSFORMATION

	Functions F(t)	Laplace Transforms f(s)
1	1	$1/s$
2	A	A/s
3	t	$1/s^2$
4	t^m	$m!/s^{m+1}$
5	Ae^{-at}	$A/(s+a)$
6	Ate^{-at}	$A/(s+a)^2$
7	$\frac{A}{a}(1-e^{-at})$	$A/s(s+a)$
8	$\frac{A}{a}e^{-(b/a)t}$	$A/(as+b)$
9	$\frac{(B-Aa)e^{-at}-(B-Ab)e^{-bt}}{b-a}$ $(b \neq a)$	$(As+B)/(s+a)(s+b)$
10	$\frac{A}{b-a}(e^{-at}-e^{-bt})$	$A/(s+a)(s+b)$
11	$e^{-at}[A+(B-Aa)t]$	$(As+B)/(s+a)^2$
12	$-\frac{1}{PQR}[P(Aa^2-Ba+C)e^{-at}+Q(Ab^2-Bb+C)e^{-bt}+R(Ac^2-Bc+C)e^{-ct}]$ $(P=b-c,\ Q=c-a,\ R=a-b)$	$(As^2+Bs+C)/(s+a)(s+b)(s+c)$
13	$A\left[\frac{1}{ab}+\frac{1}{a(a-b)}e^{-at}-\frac{1}{b(a-b)}e^{-bt}\right]$	$A/s(s+a)(s+b)$
14	$\frac{A}{a}t-\frac{A}{a^2}(1-e^{-at})$	$A/s^2(s+a)$
15	$\frac{B}{ab}-\frac{Aa-B}{a(a-b)}e^{-at}+\frac{Ab-B}{b(a-b)}e^{-bt}$	$(As+B)/s(s+a)(s+b)$
16	$\frac{B}{ab}-\frac{a^2-Aa+B}{a(b-a)}e^{-at}+\frac{b^2-Ab+B}{b(b-a)}e^{-bt}$	$(s^2+As+B)/s(s+a)(s+b)$

2. Single oral administration



$$dXg/dt = -Ka.Xg$$

$$L(Xg) = \frac{D}{(S + Ka)} \quad \text{and} \quad Xg = D.e^{-Kat}$$

The equation from the central and peripheral compartments are:

$$dX1/dt = -(K21 + K10).X1 + K21.X2 + Ka.Xg \qquad 7$$

$$dX2/dt = K12.X1 - K21.X2 \qquad 8$$

From equation 8;

$$L(X2) = \frac{K12 \,.\, L(X1)}{(S + K21)} \qquad 9$$

Substituting into equation 7;

$$S.L(X1) = -(K12 + K10).L(X1) + \frac{K12.K21.L(X1)}{(S + K21)} + \frac{Ka.D}{(S + Ka)}$$

$$\text{i.e.} \quad \frac{(S + \alpha)(S + \beta).L(X1)}{(S + K21)} = \frac{Ka.D}{(S + Ka)}$$

Where α & β are as previously defined.

Then;

$$L(X1) = \frac{Ka.D.(S + K21)}{(S + \alpha)(S + \beta)(S + Ka)}$$

From table A1, function 12;

$$X1 = \frac{Ka.D.(. - K210)}{(\alpha - \beta)(Ka - \alpha)}.e^{-\alpha t} + \frac{Ka.D.(. - K21)}{(\alpha - \beta)(\beta - Ka)}.e^{-\beta t} +$$

$$\frac{Ka.D.(Ka - K21)}{(\alpha - Ka)(Ka - \beta)}.e^{-Kat}$$

Assuming the fraction of the administered dose which is absorbed is F, then

$$X1 = \frac{Ka.D.F.(\alpha - K210)}{V1.(\alpha - \beta)(Ka - \alpha)}.e^{-\alpha t} + \frac{Ka.D.F.(\beta - K21)}{V1.(\alpha - \beta)(\beta - Ka)}.e^{-\beta t} +$$

$$\frac{Ka.D.F.(Ka - K21)}{V1.(\alpha - Ka)(Ka - \beta)}.e^{-Kat} \qquad \text{10}$$

Which may be written

$$C1 = P.e^{-\alpha t} + Q.e^{-\beta t} - (P+Q).e^{-Kat} \qquad \text{11}$$

For the metabolite

$$dXm/dt = K1/m.X1 - Kmo.Xm$$

$$(S + Kmo).L(Xm) = K1m.L(X1)$$

$$= \frac{P.V1.K1m}{(S + \alpha)} + \frac{Q.V1.K1m}{(S + \beta)} - \frac{(P + Q).V1.K1m}{(S + Ka)}$$

Thus

$$L(Xm) = \frac{P.V1.K1m}{(S + \alpha)(S + Kmo)} + \frac{Q.V1.K1m}{(S + \beta)(S + Kmo)} + \frac{(P + Q).V1.K1m}{(S + Ka)}$$

and

$$Xm = \frac{P.V1.K1m}{(Kmo - \alpha)}.(e^{-\alpha t} - e^{-Kmot}) + \frac{Q.V.K1m}{(Kmo - \beta)}.(e^{-\beta t} - e^{-Kmot}) - \frac{(P + Q).V.K1m}{(Kmo - Ka)}.(e^{-Kat} - e^{-Kmot}$$

and

$$Cm = \frac{P}{(Kmo - \alpha)}.\left(\frac{V.K1m)}{Vm}\right).(e^{-\alpha t} - e^{-Kmot}) +$$

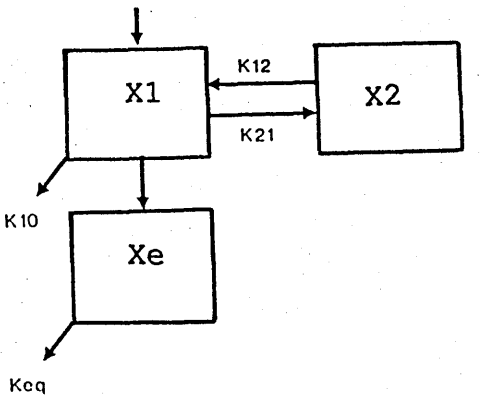
$$\frac{Q}{(Kmo - \beta)}.\left(\frac{(V.K1m)}{Vm}\right).(e^{-\beta t} - e^{-Kmot}) -$$

$$\frac{(P + Q)}{(Kmo - Ka)}.\left(\frac{(V.K1m)}{Vm}\right).(e^{-Kat} - e^{-Kmot})$$

This can be simplified to

$$Cm = R.e^{-\alpha t} + S.e^{-\beta t} + U.e^{-Kat} + T.e^{-Kmot}.$$

3. **Effect model**



$$dXe/dt = K1e.X1 - Keq.Xe$$

$$S.L(Xe) - Xe(0) = K1e.L(X1) - Keq.L(Xe)$$

$$L(Xe) = \frac{K1e}{(S + Keq)}.\ L(X1) = \frac{K1e.D}{(S + Keq)(S + Ke)}$$

i.e.

$$Xe = \frac{K1e.D}{(Ke - Keq)}.\ (e^{-Keqt} - e^{-Ket})$$

thus

$$Ce = \frac{K1e.D}{Ve(Ke - Keq)}\ (e^{-Keqt} - e^{-Ket})$$

Since $K1e.V1 = Keq.Ve$ So that

$$Ce = \frac{D.Keq}{V1(Ke - Keq)}\ (e^{-Keqt} - e^{-Ket})$$

Ce is the concentration in the effect compartment and the effect E may be related to any of the these models :

$$E = m.Ce + i \qquad \text{Linear model}$$

$$E = m.\ln Ce + i$$

$$E = \frac{Emax.Ce}{Ce50 + Ce} \qquad \text{Langmuir equation}$$

$$E = \frac{Emax.Ce^{\gamma}}{Ce50^{\gamma} + Ce^{\gamma}} \qquad \text{Hill equation}$$

PRESENTATION AT SCIENTIFIC MEETINGS

1. J.H. Ahmed, H.L. Elliott, P.A. Meredith & J.L. Reid. The pharmacokinetics of verapamil in elderly. hypertensive. Medical Research Society, Surrey, July, 1987.

2. J.H. Ahmed, H.L. Elliott, P.A. Meredith & J.L. Reid. The pharmacokinetics and pharmacodynamics of verapamil in patients with angina pectoris:effect of age. British Pharmacology Society Meeting, Liverpool, 1988.

3. J.H. Ahmed, H.L. Elliott, C.A. Howie, J. Hosie & J.L. Reid. Effects of nicardipine on the metabolic responses to food and exercise. British Pharmacology Society Meeting, London, December, 1988.

4. J.H. Ahmed, H.L. Elliott, A.C. Grant. R.S.C. Rodger & J.L. Reid. The pharmacokinetics and pharmacodynamics of nicardipine in patients with renal impairment. British Pharmacology Society Meeting, Manchester, September, 1989.

5. N. Macdonald, A. Grant, J.H. Ahmed, R.S.C. Rodger & H.L. Elliott. The impact of renal impairment on the plasma concentration of two antihypertensive drugs which are metabolised in the liver, The Renal Association, Glasgow, 1989.

6. J.H. Ahmed, J. Godden, P.A. Meredith & H.L. Elliott. The pharmacokinetics and pharmacodynamics of d-verapamil in normotensive volunteers. Caledonian Meeting, Dundee, October, 1989.

LIST OF PUBLICATIONS

1. Ahmed, J.H., Meredith, P.A., Elliott, H.L. and Reid, J.L. The influence of age on the pharmacokinetics of verapamil, Pharmacological Research, 1989 (in press).
2. Ahmed, J.H., Elliott, H.L., Meredith, P.A. and Reid, J.L. Verapamil in middle-aged and elderly patients with angina pectoris:no evidence of increased susceptibility to the cardiac effects. Journal of Cardiovascular Pharmacology, 1989 (in press).
3. Ahmed, J.H., Elliott, H.L., Hosie, J. and Reid, J.L. Effects of nicardipine on the metabolic responses to food and exercise. Journal of Human Hypertension, 1989 (in press).
4. Donnelly, R., Reid, J.L., Meredith, P.A., Ahmed, J.H. and Elliott, H.L. Factors determining the response to calcium antagonists in hypertension. Journal of Cardiovascular Pharmacology, 1988, 12:S109-S113.
5. Meredith, P.A., Elliott, H.L., Ahmed, J.H. and Reid, J.L. Age and antihypertensive efficacy of verapamil: An integrated pharmacodynamic-pharmacokinetic approach. Journal of Hypertension, 1987, S219-S221.

MANUSCRIPTS IN PREPARATION

1. Ahmed, J.H., Elliott, H.L., Grant, A.C., Rodger, R.S.C. and Reid, J.L. The pharmacokinetics and pharmacodynamics of nicardipine in patients with renal impairment.
2. Ahmed, J.H., Godden, J., Meredith, P.A. and Elliott,

H.L. The pharmacokinetics and pharmacodynamics of d-verapamil in normotensive volunteers.

3. Ahmed, J.H., Meredith, P.A. and Elliott, H.L. Age and antihypertensive efficacy of verapamil: Application of concentration-effect analysis.