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PHARMACOKINETICS OF DILTIAZEM IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS AND IN AN ISOLATED PERFUSED RAT LIVER SYSTEM

by

MUHAMMAD DELWAR HUSSAIN

A thesis

submitted to the faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **PHARMACOKINETICS OF DILTIAZEM IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS AND IN AN ISOLATED PERFUSED RAT LIVER SYSTEM** submitted by **MUHAMMAD DELWAR HUSSAIN** in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **PHARMACEUTICAL SCIENCES (PHARMACOKINETICS).**

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Dedication

To my parents and to my wife, Kity.

Abstract

Diltiazem, a tertiary amine, has a high systemic clearance mainly due to extensive hepatic metabolism. This suggests that processes or drugs that can modulate hepatic blood flow and enzyme activity have the potential to alter the kinetics of diltiazem. In humans diltiazem shows time-dependent pharmacokinetics after chronic dosing. A survey of the literature indicates that several tertiary amine drugs show time-dependent pharmacokinetics. Some of them inactivate cytochrome P450 (P450) isozymes and this inactivation may contribute to their time-dependent kinetics. The tertiary amines may be metabolized by the same enzyme(s) and this may cause mutual kinetic interactions. The objectives of this project were to study the effect of cardiopulmonary bypass (CPB) on the pharmacokinetics of diltiazem, and the mechanisms involved in diltiazem's time-dependent kinetics and interactions with other tertiary amine drugs.

A simple, specific and sensitive HPLC assay was developed for the determination of diltiazem and six of its metabolites in human plasma. This HPLC method can be used to evaluate the pharmacokinetics of diltiazem and its metabolites in patients receiving chronic diltiazem therapy.

The initiation of the CPB procedure reduced the levels of total diltiazem significantly but the levels of unbound diltiazem were not significantly changed. The plasma unbound fraction of diltiazem was doubled during the CPB and opposed the reduction in the unbound drug levels. This study indicates that supplemental diltiazem is not necessary during CPB.

The time-dependent kinetics of diltiazem in the rat were found to be mainly due to its reversible tissue binding. The liver has high affinity and large capacity for binding the drug and/or its metabolites. Lidocaine and diphenhydramine also bind to liver tissue and the binding contributes to their time-dependent kinetics. These three drugs compete for common hepatic binding sites.

Diltiazem is capable of inactivating P450 isozyme(s) involved in the *N*-dealkylation process. Both diltiazem and lidocaine inactivate similar P450 isozyme(s). Since *N*-dealkylation of diltiazem is a minor metabolic pathway in the perfused rat liver, the contribution of enzyme inactivation to diltiazem's time-dependent kinetics is minor. This is also reflected in the pretreatment study where enzyme inactivation by tertiary amines does not change the kinetic profile of diltiazem.

Coadministration of diltiazem with lidocaine or diphenhydramine resulted in a significant increase in the steady state concentration of diltiazem and its primary metabolites. The steady state extraction of lidocaine or diphenhydramine was also decreased by diltiazem.

This study indicates that the time-dependent reduction in clearance of diltiazem and several tertiary amines can be attributed to hepatic tissue binding and to inactivation of P450 isozymes. These tertiary amines may share common P450 isozymes for elimination and may interact when coadministerd.

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List of symbols and Abbreviations

AAG	α_1-acid glycoprotein
ANOVA	analysis of variance
AUC	area under the concentration *vs* time curve
AUFC	area under the flux *vs* time curve
AV	atrioventricular
b.i.d.	two times a day
ß	elimination rate constant
°C	degrees Centigrade
C_{in}	inlet concentration
C_{max}	peak drug concentration in plasma after an oral dose
C_{out}	outlet concentration
C_{ss}	steady state concentration
C_{known}	total concentration of drug and its known metabolites in the effluent
CEB	calcium channel entry blocker
Cl	clearance
Cl_H	hepatic clearance
Cl_o	oral clearance
cm	centimeter
CoA	coenzyme A
CPB	cardiopulmonary bypass

D_o	oral dose
DPH	diphenhydramine
DPH-NO	diphenhydramine *N*-oxide
DZ	diltiazem
E	extraction ratio
F	bioavailability
FDZ	plasma concentration of unbound diltiazem
FF	plasma unbound fraction
f_t	fraction of liver volume occupied by liver tissue
g	gram
g	acceleration due to gravity (9.81 m second^{-2})
GC	gas chromatography
GX	glycyl-2,6-xylidide
h	hour(s)
HPLC	high performance liquid chromatography
I.D.	internal diameter
i.p.	intraperitoneal
I.S.	internal standard
IPRL	isolated perfused rat liver
kg	kilogram(s)
K_i	inhibition constant
K_m	Michaelis Menten constant

K_{TW}	partition coefficient between the liver tissue *vs* buffer solution in the sinusoid
L	litre(s)
LID	lidocaine
μl	microlitre(s)
μm	micrometer(s)
M	molar
μM	micromolar
m	meter(s)
M1	deacetyldiltiazem
M1-NO	deacetyldiltiazem *N*-oxide
M2	*N*-demethyldeacetyldiltiazem
M4	*O*-demethyldeacetyldiltiazem
M4-NO	*O*-demethyldeacetyldiltiazem *N*-oxide
M6	*N,O*-didemethyldeacetyldiltiazem
MA	*N*-demethyldiltiazem
MB	material balance
MEGX	*N*-ethylglycyl-2,6-xylidide; monoethylglycylxylidide
min	minute(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)

n	number of observations
nCi	nanocurie
ng	nanogram(s)
nm	nanometre(s)
nmol	nanomole
NORDPH	*N*-demethyldiphenhydramine
P450	cytochrome P450
pmole	picomole
p.o.	oral
ρ	density of the liver
Q	liver perfusion rate
q.i.d.	four times a day
r	correlation coefficient
s	second(s)
SA	sinoatrial
SD	standard deviation
t	time
TDZ	plasma concentration of total diltiazem
$t_{½}$	elimination half-life
T_{max}	time at which C_{max} is reached
T_{ss}	time to reach steady state during continuous drug infusion
UV	ultraviolet

v/v	volume to volume ratio of mixtures
W	weight of the liver
yr	year(s)

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1. INTRODUCTION

Diltiazem, *cis*-(+)-3-acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one, is a benzothiazepine type calcium channel entry blocker (CEB). It was first developed as an antihypertensive drug in Japan. Diltiazem is a base (pKa = 7.7) with high lipophilicity ($\log_{10}$ P = 2.3) (Hermann and Morselli 1985). The molecule has two asymmetric carbon atoms. Among the four stereoisomers [*cis*-(+)-, *cis*-(−)-, *trans*-(+)- and *trans*-(−)-isomers], the coronary vasodilator action is highly stereoselective for the *cis*-(+)-isomer (*Sato et al. 1971*). When injected into anesthetized dogs the racemic *cis*-(±)- compound increased coronary blood flow significantly, whereas the racemic *trans*-(±)-compound was inactive. The *cis*-(+)-isomer was 1.5 to 2 times more active than the *cis*-(±)-compound. Compared to the *cis*-(±)-compound, the potency of the *cis*-(−)-isomer was very low (*Sato et al. 1971*). In another study, Nagao *et al.* (*1972*) also found that the *cis*-(+)-isomer was the most powerful vasodilator for both coronary and femoral arteries and this effect was followed by *cis*-(±)-, *cis*-(−) and *trans*-(±)-isomer in decreasing order. The *cis*-(−)-diltiazem is 20-30 times less potent than the *cis*-(+)-diltiazem in calcium channel blocking action (*Nagao et al. 1982*). Also compared to *cis*-(+)-diltiazem, the *cis*-(−)-isomer has less inhibition of tissue free fatty acid accumulation (*Nasa et al. 1990*). The marketed formulation contains the *cis*-(+)-isomer only.

In the human, it has been found that inactive isomers of some but not all 2-arylpropionic acids (2-APA) such as ibuprofen, fenoprofen and benoxaprofen undergo

in-vivo metabolic chiral inversion to their pharmacologically-active antipodes (*Jamali et al. 1992*). The inactive *R*-ibuprofen undergoes highly stereospecific conversion to its coenzyme A (CoA) thioester *in vivo*. Enzymatic loss of the α-methine proton gives a symmetrical ibuprofen enoyl-CoA, a tautomer intermediate resulting in the transformation of *R*-ibuprofen CoA to its *S* counterpart and subsequent hydrolysis gives *S*-ibuprofen (*Sanins et al. 1991*). *R*-Ibuprofen and *R*-fenoprofen are stereoselectively incorporated into adipose tissue in the form of "hybrid triglycerides" and provide proof for the intermediate CoA derivatives of these drugs (*Williams et al. 1986, Sallustio et al. 1988*). The mechanism for *in-vivo* chiral inversion discussed above suggests that a carboxyl group with a exchangeable α-methine proton is necessary if it is to be a substrate for enzymes of the fatty acid ß-oxidation complex. Thus it seems unlikely that diltiazem, containing chiral centres on the rigid nonplanar benzothiazepine ring and no carboxyl group attached to chiral centre, will undergo metabolic chiral inversion in the body. Until now no *in vivo* study has been reported that explored the possibility of metabolic chiral inversion of diltiazem. However, in one *in vitro* study, no racemization of diltiazem (1% solution in 0.45 M HCl or buffer, at pH 0.45 to 6.1) was observed even after heating at 70°C for an extended period of time (*Chafetz and Shah 1991*).

1.1 Calcium and Calcium Channels

Intracellular free Ca^{2+} concentration plays an important role as a messenger for cellular functions in both normal and pathological conditions. In resting myocardial and smooth muscle cells, the intracellular concentration of free Ca^{2+} is $\sim 5 \times 10^{-8}$ M or less and

rises to ~5 x 10^{-7} M during cell excitation (*Triggle 1990a*). At this higher free concentration, Ca^{2+} binds to troponin or calmodulin (binding proteins). This binding allows interaction between the myosin bridge and the actin filament in the presence of ATP and results in muscular contraction. When intracellular free Ca^{2+} concentration is reduced, Ca^{2+} is dissociated from the binding proteins. This causes the actin-myosin cross-link to break and subsequent muscular relaxation (*Braunwald 1982*). Thus the isometric myocardial systolic and diastolic tensions are directly related to the myoplasmic free Ca^{2+} concentration. To control the level of intracellular free Ca^{2+}, a set of processes is coordinated by the cell. These processes in myocardium are represented schematically in Figure 1.1 (*Braunwald 1982*). The main sources and storehouses for intracellular Ca^{2+} mobilization and sequestration are the infoldings of the surface membrane (the transverse tubular system), the mitochondria and particularly the sarcoplasmic reticulum. The initial influx of Ca^{2+} across the transverse tubular membrane through channels (mechanism 1A and 1B) triggers the secondary and much larger release of Ca^{2+} from the sarcoplasmic reticulum, followed by activation of the contractile system. Schneider and Chandlier (*1973*) proposed that a "voltage sensor" is located in the transverse tubular membrane and controls the release of calcium from the sarcoplasmic reticulum. In response to changes of transverse-tubular potential, the voltage sensor undergoes molecular rearrangement and controls calcium flow from the sarcoplasmic reticulum. Other pathways of movement of Ca^{2+} across sarcolemma includes bidirectional Na^{+}-Ca^{2+} exchange (mechanism 2), Ca^{2+} leak pathways (mechanism 6) and extrusion of Ca^{2+} (mechanism 3). Intracellular Ca^{2+} can be taken up

into the lumen of sarcoplasmic reticulum (mechanism 4) and other intracellular structures such as mitochondria (mechanism 5).

The sarcolemmal channels represent a very selective and important pathway of Ca^{2+} influx into excitable cells (*Bean 1989, Triggle 1990b*) and are termed as "calcium channels". Voltage-dependent calcium channels mediate the influx of Ca^{2+} in response to membrane depolarization and play a vital role in excitation-contraction coupling of cardiac and smooth muscle (*Hagiwara and Byerly 1981*). In the receptor-operated calcium channels, the receptor and the channel may belong to the same protein(s) or the receptor and the channel may be physically separate and connected through cytosolic or membrane messengers (*Triggle et al. 1989*). The indication of a receptor-operated voltage-independent channel in the vascular smooth muscle is weak (*Benham and Tsien 1987*). The beta-adrenergic agonist induced stimulation of the myocardium still needs voltage stimulation to open the channel (*Schneider and Sperelakis 1975*). Thus the voltage-dependent channels may be considered the only type of calcium channels in the myocardium.

The influx of positively charged calcium ions is associated with an electric current, called the calcium current. The rates of activation and inactivation of this inward calcium current are slower than those of the fast inward current produced by influx of Na^{+} (*Carmeliet 1980*) and thus the calcium current is also known as "slow inward current". The calcium current plays a critical role in the generation and maintenance of the plateau of the cardiac action potential, allowing more cytosolic free Ca^{2+} to trigger contraction (*Fozzard 1983*).

Sinoatrial (SA) pacemaker cells and atrioventricular (AV) automatic cells have slowly rising action potentials and a reduced rate of conduction (*Braunwald 1982*). Both automaticity and conduction in SA and AV node are largely mediated by transmembrane influx of Ca^{2+} through calcium channels (*Wit and Cranefield 1974, Zipes and Fischer 1974*). Thus the calcium channel is not only involved in vascular and cardiac muscle contraction but is also involved in conduction of impulses and in pacemaker activity of the nodal tissue. Processes that modify calcium current will have important effects on SA and AV nodes.

The calcium channels are divided into three subtypes, termed L, T, and N channels based on their voltage threshold for activation, conductances and inactivation characteristics, (*Bean 1989, Fox et al. 1989, Triggle 1990a*). The L-type channels, characterized by high threshold, large conductance and slow inactivation (long-lasting, or L type), are involved in maintenance of the action potential plateau. The T channels have low threshold and low conductance. They are rapidly inactivated (transient, or T type) and are involved in pacemaking and trigger functions. The N (nervous) channels are found in neurons, have properties in between those of the L and T channels, and are involved in release of neurotransmitters from nerve terminals (*Carmeliet 1988, Miller 1987, Wagner et al. 1988*).

The predominant type of voltage-dependent calcium channel in the heart and in muscle cells is the L type channel (*Catterall et al. 1989, Hosey et al. 1989*). These channels appear to be present in high density in skeletal muscle transverse tubule membranes. The kinetics of opening and closing of L channels in cardiac cells are quite

different from those in skeletal muscle. Thus subtypes of L channels are possible and structural differences may be responsible for the observed differences in properties (*Hosey et al. 1989*).

1.2 Calcium Channel Entry Blockers

Although specific drugs may interact with each of the calcium control processes shown in fig. 1.1., in practice, only one class of drugs interacting at the plasmalemmal voltage-dependent calcium channels is therapeutically useful (*Triggle 1990a*). Fleckenstain (*1977*) found that some compounds can selectively block the excitation-contraction coupling in heart muscle. This blocking effect can be overcome by agents that increase the supply of Ca^{2+}, and by Ca^{2+} itself. Because of their specific antagonism to the movement of extracellular Ca^{2+}, they were called calcium channel entry blockers or calcium antagonists. Calcium channel entry blockers are chemically heterogeneous and include analogs of benzothiazepine, phenylalkylamine and dihydropyridine.

The L type calcium channel which is responsible for calcium entry during the plateau of the action potential is sensitive to diltiazem, nifedipine and verapamil, the three prototypes of CEBs (*Triggle 1990a*). These three agents, with different molecular structures, bind at an allosterically linked set of sites on a major protein of the L class of vascular and myocardial calcium channels. These sites represent the active part of the calcium channel and interaction at these binding sites blocks channel function (*Triggle 1990b*).

Although diltiazem, nifedipine and verapamil have clinical effects related to inhibition of vascular contraction (antihypertensive and antianginal effects), only diltiazem and verapamil have a clinically relevant inhibitory effect on the AV node (inhibition of supraventicular tachycardia) (*Soward et al. 1986, Triggle 1990b*). Both verapamil and diltiazem prolongs the refractory period in the AV node by a direct action on the calcium channels in the AV node. This effect is clinically important and forms the basis for the use of verapamil and diltiazem in the prevention of recurrences of supraventicular tachycardia and in the slowing of ventricular response during atrial flutter and fibrillation (*Braunwald 1982, Nademanee and Sing 1988*). The CEBs can reach the receptor site of the calcium channel either through the open channel or through the bilipid membrane to reach the receptor (*Sanguinetti and Kass 1984*). Verapamil and diltiazem are in charged form at physiological pH (7.4) and since lipid penetration is low, entry is *via* the open state of the calcium channel. This explains why verapamil and diltiazem are frequency-dependent. The neutral, uncharged lipophilic dihydropyridines can reach the calcium channel associated receptor by penetration of lipid bilayer and are voltage dependent (*Triggle 1990b*). Thus frequency-dependent verapamil and diltiazem would enter rapidly through the increased opening of the AV nodal calcium channels to exert their inhibitory effects on the calcium channel (*Sanguinetti and Kass 1984*).

1.3 Pharmacology of Diltiazem

The pharmacological properties of diltiazem and its therapeutic efficacy have been reviewed by Chaffman and Brogden (*1985*) and Buckley *et al.* (*1990*).

Diltiazem inhibits ion-control gating mechanisms of the Ca^{2+} channel and interferes with the intracellular Ca^{2+} influx and subsequent Ca^{2+} release from the sarcoplasmic reticulum (*AHFS drug information, 1991*). Thus diltiazem inhibits the Ca^{2+} dependent contractile processes of vascular smooth muscle thereby dilating the main coronary and systemic arteries (*Buckley et al. 1990*). By inhibiting higher calcium flux in the ischemic cardiac tissue, diltiazem conserves high energy phosphates and thus protects the cardiac tissues at the cellular level (*Itoh et al. 1987, Takeo et al. 1988*).

1.3.1 Use of Diltiazem

Diltiazem, due to its inhibition of Ca^{2+} influx into the cardiac and vascular cells, is an effective and well-tolerated treatment for stable angina and angina due to coronary artery spasm (*Chaffman and Brogden 1985*). Several mechanisms contribute to the antianginal effect of diltiazem. These include coronary vasodilation, decreased myocardial work (decreased afterload, heart rate and myocardial contractility), improved subendocardial perfusion and protection against Ca^{2+} overload. Since diltiazem is a coronary vasodilator it is especially effective in myocardial ischemia due to coronary spasm or vasoconstriction. Diltiazem is particularly useful in relieving coronary vasospasm which usually is superimposed on vessels already critically narrowed by atherosclerosis (*Flaim and Zelis 1981a*). Inhibition of spontaneous and induced coronary artery spasm results in increased myocardial oxygen supply (*AHFS drug information 1991, Chaffman and Brogden 1985*). Experimentally, when coronary vasoconstriction is relieved by CEBs, blood flow increases to the subendocardial zones (*Thaulow et al.*

1987). Dilation of systemic arteries by diltiazem results in a decrease in total peripheral resistance, a decrease in systemic blood pressure, a decrease in the afterload of the heart and thus a decrease in myocardial oxygen demand. The reflex tachycardia appears to be suppressed by diltiazem acting at the SA node. The double effect (heart rate x systolic pressure) generally declines after intravenous diltiazem, indicating a reduction in myocardial oxygen demand (*Joyal et al. 1986, Legrend et al. 1984*). The dominant mechanism of the antianginal effect of diltiazem seems to be the reduction of myocardial oxygen demand. Diltiazem improves the function of ischemic segments that are not dyskinetic after acute coronary occlusion (*Clozel et al. 1983*). There is also evidence that diltiazem has a protective effect during myocardial ischemia, reduces arrhythmias associated with ischemia and may protect the ischemic myocardium from calcium-induced damage during reperfusion (*Flaim and Zelis 1981b, Krukenkamp et al. 1986*). The effective concentration of diltiazem for its anti-ischemic action ranges from 50 to 200 ng/ml in plasma (*Godet et al. 1987, Sugihara et al. 1984*).

Diltiazem is used in the management of mild to moderate hypertension. In addition to its lowering of blood pressure, diltiazem decreases heart rate by 8-10%. The double effect is shown both at rest and during exercise (*Buckley et al. 1990*). This drug has been used as monotherapy or in combination with other classes of antihypertensive agents. In monotherapy, diltiazem compares well with ß-blockers. Diltiazem may be more effective in certain patient groups, such as elderly and black, probably due to low serum renin level and low serum Ca^{2+} (*Buckley et al. 1990, Triggle 1990b*). It may have a special role in the therapy of hypertensive patients with physical and mental strain

(*Schmieder et al. 1987, Szlachcic et al. 1987, Trimarco et al. 1984*). Diltiazem may revert left ventricular hypertrophy (*Frohlich 1987*). However, the targeted blood pressure may not always be reached during monotherapy so that drug combinations may be required. The combination of a calcium channel blocker and a ß-adrenoceptor blocker is widely used in the management of ischemic heart disease and hypertension (*Brouwer et al. 1985, Chaffman and Brogden 1985*). Johnston *et al.* (*1985*) suggested propranolol-diltiazem for combined therapy rather than propranolol-nifedipine or propranolol-verapamil because of its low incidence of adverse clinical effects. A combination of diltiazem and hydrochlorothiazide lowers blood pressure to a greater extent than either drug alone (*Weir et al. 1989*).

Oral diltiazem is effective in preventing the recurrence of supraventicular tachycardia (*Jauernig et al. 1983, Yeh et al. 1983*) and in controlling the ventricular response to atrial fibrillation/flutter (*Roth et al. 1986, Steinberg et al. 1987*). As mentioned earlier, calcium current plays a prominent role in depolarizing and pacemaking functions of both SA and AV nodes. The sinus node is inhibited by all three major CEBs, but the magnitude of this effect on the heart rate depends, in part, on reflex sympathetic stimulation. The stimulation is normally most pronounced with nifedipine and least prominent with diltiazem (*Triggle 1990b*). Diltiazem inhibits the AV node and increases both nodal conduction time and nodal refractoriness at the AV node (*Nademanne and Sing 1988*). These changes explain why diltiazem is clinically effective in supraventricular tachycardia and atrial fibrillation. Diltiazem is not directly effective

against atrial flutter, but is beneficial in slowing the contraction rate of the ventricles (*Buckley et al. 1990*).

1.3.2 Side Effects of Diltiazem

Among the available calcium antagonists, diltiazem seems to be the best tolerated drug (*Dustan 1989, Russel 1988*). The major side effects of diltiazem can be anticipated from its pharmacological actions. Side effects secondary to vasodilation such as headache, flushing, peripheral oedema and hypotension may occur (*Buckley et al. 1990*). Like verapamil, diltiazem has a substantial inhibitory effect on the cardiac conduction system. The resting heart rate is slightly reduced by diltiazem, especially in patients with SA node disease. Due to its slowing action on the AV node conduction, diltiazem may rarely cause second- or third-degree AV block (*Chaffman and Brogden 1985*).

1.4 Pharmacokinetics of Diltiazem

1.4.1 Absorption

Diltiazem is rapidly absorbed after oral administration to humans (*Hoglund and Nilsson 1989a, Piepho et al. 1982, Rovei et al. 1980, Stone et al. 1980*). The time to reach peak plasma concentration, T_{max}, for diltiazem after an oral dose of a solution form is 38 $\pm$ 6 min in healthy subjects (*Ochs and Knuchel 1984*). T_{max} ranged from 2 to 4 h and peak plasma concentration, C_{max}, ranged from 42 to 72 ng/ml after administration of a single 60 mg conventional tablet (*Caille et al. 1991, du Souich et al. 1990, Hermann*

et al. 1983, Kinney et al. 1981, Smith et al. 1983). T_{max} and C_{max} after single administration of a 120 mg slow release capsule were 7.1 ± 0.2 h and 99.0 ± 9.8 ng/ml, respectively (*du Souich et al. 1990*). Bioavailability from conventional tablets (Cardizem, Marion Merrel Dow) ranged between 30 ± 5% and 42 ± 18% (*Dubruc et al. 1985, Hermann et al. 1983, Smith et al. 1983*). Absolute bioavailability from oral sustained release tablets (Tanabe, Seiyaku, Osaka, Japan) was 33 ± 4% (*Ochs and Knuchel 1984*). Thus, although the rate of absorption differs among oral dosage forms, such as immediate and slow release tablets or capsules, absolute bioavailability is not changed (*Buckley et al. 1990*). The bioavailability averages around 35% after a single oral dose and increases with multiple doses (*Smith et al. 1983*). Since diltiazem is extensively metabolized by liver, the low systemic bioavailability has been attributed mainly to extensive first-pass hepatic metabolism (*Hermann et al. 1983, Ochs and Knuchel 1984, Smith et al. 1983*). Animal studies have shown that 95% of radiolabeled diltiazem was absorbed after oral administration (*Meshi et al. 1971, Piepho et al. 1982*). Based on radioactivity in urine after intravenous (20 mg diltiazem solution with 1.85 MBq ^{14}C-diltiazem) and oral (60 mg or 120 mg diltiazem solution with 1.85 MBq ^{14}C-diltiazem) dosing in the human, Hoglund and Nilsson (*1988, 1989a, 1989b*) suggested that diltiazem was almost completely absorbed after oral administration. Excretion of the tracer in the urine (mean 71-73% after 60 and 120 mg oral diltiazem) was not different from the results found (71 ± 8%) after intravenous administration. The total amount of tracer excreted in urine and feces within 120 h was ~87% on both occasions (*Hoglund and Nilsson 1989a*). These radioactivity data do not provide absolute proof

that diltiazem is absorbed completely intact across the gastrointestinal (GI) tract because it does not rule out the possibility that diltiazem undergoes first pass GI tract metabolism.

Large interindividual differences is observed in the C_{max} and area under the plasma concentration-time curve (AUC) of diltiazem (*Bianchetti et al. 1991, Kinney et al. 1981, Morselli et al. 1979, Zelis and Kinney 1982*). This may be explained by variable first-pass elimination due to individual variations in the functional status of hepatic metabolic enzymes (*Bianchetti et al. 1991, Hermann et al. 1983*). Food does not affect the bioavailability of diltiazem administered as a conventional tablet or in a slow release formulation (*du Souich et al. 1990*).

1.4.2 Distribution

Diltiazem distributes extensively to tissues and organs. After intravenous administration of ^{14}C-diltiazem to rats, high radioactivity was found in the liver, lung, kidney, cardiac muscle, adrenal gland, pituitary gland, pineal body, intestinal wall and Harder's gland (*Nakamura et al. 1987*). After oral administration of ^{14}C-diltiazem, radioactivity in various tissues peaked at 15 to 30 min (*Piepho et al. 1982*). The high radioactive levels of diltiazem in the liver, interlobular bile ducts and gall bladder, and its rapid metabolism (only 11 percent of the hepatic radioactivity was due to the parent compound 5 min after the intravenous dose) emphasize the role of the hepatic system in the elimination of diltiazem (*Piepho et al. 1982*).

About 80% of diltiazem is bound to human plasma proteins (*Boyd et al. 1989, Morselli et al. 1979, Piepho et al. 1982*). Of the protein bound fraction, 35-40%

involves albumin and the remainder binds to α_1-acid glycoprotein (AAG) (*Belpaire and Bagaert 1990, Morselli et al. 1979, Pieper 1984a*). Diltiazem may also bind to lipoproteins (*Kwong et al. 1985*). The plasma protein binding of diltiazem increases with increasing AAG and the variability in protein binding in patients can be explained by AAG concentrations (*Belpaire and Bogaert 1990, Kwong et al. 1985, Nakamura et al. 1987, Pieper 1984a*). In the clinically observed concentration range (0.1 to 1 μg/ml), diltiazem binding is independent of the drug concentration (*Belpaire and Bogaert 1990 and Pieper 1984a*).

Diltiazem has a large volume of distribution that can be related to its high lipophilicity (*Hermann and Morselli 1985*). The volume of distribution is approximately 5-11 L/kg in healthy subjects (*Hermann et al. 1983, Ochs and Knuchel 1984*) and 4-7 L/kg in patients with angina (*Clozel et al. 1984*).

Diltiazem is excreted into human milk, apparently in concentrations approximately equal to the maternal serum concentrations (*Okada et al. 1985*). After intravenous administration of ^{14}C-diltiazem to the pregnant rat, the placental radioactivity was approximately four times that of maternal plasma, while the concentration in the fetus and amniotic fluid did not attain that found in maternal organs (*Piepho et al. 1982*).

After oral administration of diltiazem to healthy subjects, its plasma profile can be adequately described by a two compartment open model (*Hermann et al. 1983, Hoglund and Nilsson 1989a, 1989b, Rovei et al. 1980*). However, some authors (*Kolle et al. 1983, Ochs and Knuchel 1984*) have described the pharmacokinetic profile by a three compartment open model. Kolle *et al.* (*1983*) estimated a terminal γ-phase

although they did not observe this phase after intravenous infusion. A weighting factor equal to the reciprocal of the observed terminal concentrations was used by the authors. Thus the lower concentrations which were close to the sensitivity limit of the assay (higher degree of uncertainty), were given more weight in the calculations. Only a minor fraction of diltiazem is eliminated during the γ-phase and, compared to the total elimination process, the contribution of this phase is very small (*Hermann and Morselli 1985, Hoglund and Nilsson 1988*). In practice, a two compartment open model is more useful and more reliable in routine work.

1.4.3 Metabolism

The metabolism of diltiazem has been studied both in humans and animals (*Meshi et al. 1971, Nakamura et al. 1987, Rovei et al. 1980, Sugihara et al. 1984, Yeung et al. 1990*). Diltiazem is extensively metabolized in man; less than 5% of the orally administered dose is excreted in urine as unchanged drug (*Rovei et al. 1980, Yeung et al. 1990*). The proposed metabolic pathways in humans are shown in Figure 1.2. The four phase I metabolic pathways in humans are deacetylation, *N*-demethylation, *O*-demethylation and *N*-oxidation (*Hussain et al. 1992, Sugihara et al. 1984, Yeung et al. 1989*). Oxidative deamination is also reported to be one of the metabolic pathways of diltiazem in human (*Sugawara et al. 1988*). Some of the metabolites are converted in part to glucuronides and/or sulfates (*Sugihara et al. 1984*). The major metabolites of diltiazem in man are *N*-demethyldiltiazem (MA), deacetyldiltiazem (M1) and *N*-demethyldeacetyldiltiazem (M2) (*Hussain et al. 1992, Sugihara et al. 1984, Yeung et al.*

1989, 1990). Plasma concentrations of these metabolites are about 30-50%, 10-30% and 10-20%, respectively, of the parent drug (*Buckley et al. 1990*). Other metabolites, *O*-demethyldeacetyldiltiazem (M4), *N,O*-didemethyldeacetyldiltiazem (M6) and deacetyldiltiazem *N*-oxide (M1-NO) are also present in human plasma but in lower quantities (*Hussain et al. 1992, Yeung et al. 1989, 1990*). It is interesting to note that the metabolic pattern in man is qualitatively similar to that found in rats (*Meshi et al. 1971*).

Although the oxidative metabolism of diltiazem is well established, only one study has been performed to identify the involvement of cytochrome P450IIIA isozymes in the N-demethylation of diltiazem in human and rabbit (*Pichard et al. 1990*).

The metabolites of diltiazem contribute to its pharmacological actions. The ranking of the coronary-vasodilating activity of the metabolites in decreasing order is M1, MA, M2, M4 and M6. The coronary-vasodilating abilities of M1 and MA are 50% and 20%, respectively, that of diltiazem (*Rovei et al. 1980, Sugihara et al. 1984, Yabana et al. 1985*). M1 produces a hypotensive action as potent as diltiazem does, and MA produces 50% of the action of diltiazem (*Schoemaker et al. 1987, Yabana et al. 1985*). M1, M4 and M6 are more potent than diltiazem in inhibiting platelet aggregation activity (*Kiomoto et al. 1983*). Also M1 and M4 are more potent than diltiazem in inhibiting the uptake of adenosine in blood cells (*Yeung et al. 1991a*). Some of the metabolites of diltiazem, such as M1, MA and M2, have longer half-lives than the parent drug and thus they may contribute to the overall therapeutic and adverse effects of diltiazem (*Boyd et al. 1989, Yeung et al. 1990*). The plasma protein binding properties of M1 and MA are

similar to that of diltiazem (*Boyd et al. 1989*) and the presence of the metabolite M1 does not affect the protein binding of the parent drug (*Piepho et al. 1982*).

1.4.4 Excretion

After intravenous administration of ^{14}C-diltiazem to healthy volunteers, 87% of the total radioactivity was excreted in the first 120 h, 71.1% in urine and the remaining amount in feces (*Hoglund and Nilsson 1988*). Further, more than 80% of the amount excreted in urine was conjugated. The total amount of diltiazem, MA and M1 excreted unchanged in 24 h urine following 120 mg diltiazem administration to healthy adults was 2.3%, 3.2% and 0.23% respectively (*Boyd et al. 1989*). Diltiazem also appears in the bile, undergoes enterohepatic circulation and is excreted i feces (*Meshi et al. 1971, Piepho et al. 1982, Smith et al. 1983*).

The mean plasma elimination half-life of diltiazem generally ranges from 3 to 6 h (*Boyd et al. 1989, du Souich et al. 1990, Hermann et al. 1983, Hoglund and Nilsson 1989a, Smith et al. 1983, Tawashi et al 1991, Zelis and Kinney 1982*) but higher values have been obtained using a three-compartment open model (*Kolle et al. 1983, Ochs and Kunchel 1984*).

The total body clearance of intravenous diltiazem ranges from 11.5 to 21.3 ml/min/kg in healthy subjects (*Hermann et al. 1983, Ochs and Kunchel 1984*) suggesting that the drug is eliminated at a rate that is dependent on hepatic blood flow. Thus, factors that change hepatic blood flow such as age, activity and certain interacting drugs may influence the pharmacokinetics of diltiazem. Hepatic blood flow rate changes

throughout the day and the time of administration of diltiazem affects its pharmacokinetics (*Kelly et al. 1991*).

1.4.5 Pharmacokinetics in the Elderly and in Disease State

The elimination half-life of diltiazem was longer in the elderly patients than in younger ones after 60 mg of diltiazem b.i.d. but AUC and C_{max} values, and urinary excretion of unchanged diltiazem were not changed (*Morselli et al. 1979*). These data suggest that bioavailability is not changed but that the volume of distribution may be increased in the elderly. Since diltiazem is extensively metabolised by liver, hepatic disease is expected to change the pharmacokinetics of diltiazem. In liver cirrhosis, clearance of diltiazem was decreased and elimination half-life and bioavailability of diltiazem were increased (*Echizen and Eichelbaum 1986, Kurosawa et al. 1990*). Coronary artery disease or renal disease do not affect the pharmacokinetics of diltiazem (*Hermann and Morselli 1985*). Tawashi *et al.* (*1991*) also found that the terminal elimination phase of diltiazem is not affected by chronic renal failure. This is most likely due to the lesser importance of the kidney in the elimination of unchanged diltiazem.

1.4.6 Plasma Concentrations and Effects

Establishment of simple relationships between dose and plasma concentration or between plasma concentration and effect, following diltiazem administration, are difficult due to large intrasubject and intersubject variability in plasma concentration, presence of active metabolites and age related difference in response (*Kelly and O'Malley 1992*).

However, there is evidence for a therapeutically effective concentration range for diltiazem, and the minimum effective concentration for angina pectoris and hypertension has been suggested to be 100 ng/ml (*Morselli et al. 1979, Joyal et al. 1986*).

1.5 Pharmacokinetic Interactions

Diltiazem is often administered in combination with a variety of other drugs and the potential for both pharmacodynamic and pharmacokinetic drug interaction exists. Several pharmacokinetic drug interactions have been reported in the literature that result from interferences at the metabolic level. Diltiazem is a potent inhibitor of hepatic oxidation by cytochrome P450 (P450) (*Renton 1985*). In healthy volunteers, diltiazem decreases the clearance and increases the elimination half-life of antipyrine, an agent used to assess hepatic oxidative metabolism (*Abernethy et al. 1988, Bauer et al. 1986, Carrum et al. 1986*). Diltiazem did not affect the hepatic blood flow and the volume of distribution of antipyrine (*Bauer et al. 1986*). The drug interaction of antipyrine with diltiazem is apparently due to inhibition of hepatic oxidation. This inhibition is selective in that the 3-hydroxylation pathway for antipyrine was inhibited but the 4-hydroxylation and the O-demethylation pathways were not inhibited (*Abernethy et al. 1988*) suggesting specific P450 isozymes are involved. Diltiazem inhibits warfarin disposition in humans in a stereospecific manner. Also the inhibition is regiospecific; R-6 and R-8 hydroxywarfarin are selctively inhibited. Partially purified human $P450_7$ is likely to be the isozyme inhibited by diltiazem (*Abernethy et al. 1991*). Unfortunately the specific human P450 forms that mediate formation of the various oxidized warfarin metabolites

have not been identified and the data that was accumulated cannot be reliably converted to the standard P450 nomenclature (*Nerbert et al. 1989*). The description of the specificity of the inhibitory interaction was based on the nomenclature described by Wang *et al.* (*1983*), who did not provide amino acid sequences for the P450 forms. In human and rabbit, isozymes of the P450IIIA subfamily are the major enzymes involved in the *N*-demethylation of diltiazem (*Pichard et al. 1990*). Accordingly, drugs that are specific substrates, or inducers of these P450s are likely to influence the pharmacokinetics of diltiazem.

Diltiazem increased cyclosporin blood concentrations by 25-100% in renal allograft patients. This may be due to interference of P450-mediated cyclosporin metabolism (*Buckley et al. 1990*). Based on experiments with microsomes from human liver, Brockmoller *et al.* (*1990*) reported that the effect of diltiazem was due to noncompetitive inhibition of cyclosporin metabolism by diltiazem. Cyclosporin, a specific substrate of P450IIIA, competitively inhibited diltiazem *N*-demethylase in human and rabbit (*Pichard et al. 1990*). Diltiazem also increased plasma digoxin levels due to impairment of hepatic clearance of the cardiac glycoside (*North et al. 1986, Rameis et al. 1984*). Diltiazem interfered with hepatic metabolism of propranolol and metoprolol but did not affect the AUC or elimination half-life of atenolol (*Tateishi et al. 1989*). The lack of effect on atenolol may be because it is mainly excreted unchanged in the urine (*Cruickshank 1980*). Although coadministration of diltiazem and propranolol caused an increase in bioavailability and half-life, and a decrease in oral clearance of propranolol, diltiazem was not affected by the combination (*Dimmitt et al. 1991, Etoh et al. 1983*).

Diltiazem increased the AUC (relative bioavailability) of imipramine by 30% as compared with placebo (*Hermann et al. 1992*). Inhibitory interactions of diltiazem in hepatic drug oxidation that may have clinical significance have been described with carbamazepine (*Al-Humayyd 1990, Brodie and MacPhee 1986, Eimer and Carter 1987*), nifedipine (*Frishman et al. 1988, Tateishi et al. 1989, Toyosaki et al. 1988*) and encanide (*Kazierad et al. 1989*).

Plasma concentrations of diltiazem and M1 were significantly increased by cimetidine (*Winship et al. 1985*). Amiodarone also significantly increased diltiazem plasma concentration (*David and Bialer 1989*). In contrast, diazepam decreased the plasma diltiazem level probably by reducing its absorption when these drugs were given together (*Morselli et al. 1979*).

Diltiazem is not displaced from its plasma binding sites either in presence of its metabolite, M1, or digoxin (*Piepho et al. 1982*). Acidic drugs that are mainly bound to albumin, *e.g.* phenylbutazone, indomethacin, hydrochlorothiazide, salicylic acid or warfarin, did not appear to interfere with the plasma protein binding of diltiazem (*Morselli et al. 1979, Piepho et al. 1982*). These data suggest that another plasma protein, AAG, may play a major role in diltiazem binding. In an *in vitro* experiment, the addition of 5 to 100 μg/ml of lidocaine, disopyramide, bupivacaine or quinidine, which bind to AAG, decreased the serum protein binding of diltiazem (*Belpaire and Bogaert 1990*). Also diltiazem has been found to increase the propranolol-free fraction in serum (*Pieper 1984b*). Thus, these basic drugs and diltiazem share common binding sites on serum proteins, probably on AAG.

1.6 Determination of Diltiazem and its Metabolites in the Biological Fluid

Various methods, including both high-performance liquid chromatography (HPLC) and gas chromatography (GC), have been reported for the quantification of diltiazem and its metabolites in plasma. The major problem with these assays is that most of them can only quantify diltiazem and two of its metabolites with low sensitivity (around 25 ng/ml). In order to study diltiazem metabolism and kinetics in man and animals a more sensitive assay is required which is capable of measuring diltiazem and all of its known metabolites. This aspect becomes more important in kinetic studies of drug-drug interactions where mass balance may be achieved by measuring all the known metabolites and a lack of sensitivity may create problem in characterizing metabolite kinetics. Recently, Yeung *et al.* (*1989*) reported an HPLC method capable of quantifying diltiazem and six of its metabolites. Sample preparation used in the method was tedious, requiring multiple extraction processes, and the chromatograms were not completely free of interfering peaks. Metabolites were not baseline separated and the assay precision had a variation up to 60%, even though 2 ml of plasma was used. A simple and more sensitive assay is desirable for the determination of diltiazem and its known metabolites.

1.7 Stability of Diltiazem and Metabolites

The acetate ester group in diltiazem can be hydrolysed and the rate of deacetylation is pH dependent. Maximum stability of diltiazem is observed at pH ~3.5; the hydrolysis increases with either an increase or decrease in pH (*Chafetz and Shah 1991*). These data suggests that *in situ* degradation of diltiazem in physiological fluids

may contribute to the high total body clearance of diltiazem. The metabolite MA still carries the acetate ester group and the formation of M2 from MA *via* hydrolysis is possible. Also sample collection, storage and treatments during analysis may effect the stability of diltiazem and its metabolites. All of these factors could lead to errors in the determination of drug and metabolite levels in biological fluids and in calculating pharmacokinetic parameters. For example, some authors have reported that M1 is the major metabolite of diltiazem after oral administration (*Chaffman and Brogden 1985, Rovei et al. 1980, Smith et al. 1983*) while authors of other studies have claimed that MA is its predominant metabolite (*Hoglund and Nilsson 1989a, Hussain et al. 1992, Montanat and Abernethy 1987*).

Several studies have been conducted to address the stability of diltiazem and its metabolites under different conditions. The half-lives of hydrolytic degradation of diltiazem at 37°C in whole blood, plasma and gastric fluid are 27 h, 88 h and 153 h respectively. Thus, nonenzymatic hydrolytic degradation in the biological fluids makes a minimal contribution to the clearance of the drug (*McLean et al. 1991)*. To achieve accurate determination of diltiazem, M1 and MA, blood samples must be centrifuged immediately after collection or kept on ice for up to 1 h (*Bonnefous et al. 1992*). At 4°C, no significant degradation of diltiazem, MA, M1, M2 or M4 occurs in plasma up to 24 h (*Dube et al. 1988*). In plasma, these compounds and M6 are stable for 8 weeks when stored at -20°C (*Yeung et al. 1991b*). Some authors recommended that analysis of frozen (-20°C) plasma samples be performed within 2-5 weeks after collection (*Bonnefous et al. 1992, Caille et al. 1989, Dube et al. 1988*). Storage at -70°C to -80°C

may improve the stability of diltiazem and its metabolites in plasma (*Bonnefous et al. 1992, Yeung et al. 1991b*).

In 0.01 N HCl solutions, diltiazem, M1 and MA showed no degradation for periods up to 48 h at room temperature and on exposure to daylight. In water or buffer, both at a pH near 6, the temperature of incubation had little effect on DZ stability (*Caille et al. 1989*). There was no degradation of diltiazem in plasma or buffer (pH 7.4) stored at room temperature for 48 h, while MA levels decreased significantly in plasma, but not in buffer, after 24 h. The reduction of MA was associated with an increase of M2. This suggested that the degradation of MA in plasma may be mediated by a carboxylesterase (*Caille et al. 1989*).

1.8 Cardiopulmonary Bypass

During cardiac surgery, such as coronary artery bypass graft operation and corrective surgery for heart valves, the heart's pumping action is stopped and an artificial device, the cardiopulmonary pump-oxygenator, is used to substitute for cardiac and pulmonary functions. After heparinization, the patient blood from the caval veins is led to the oxygenator/heat exchanger and is pumped to the ascending aorta usually by means of a non-pulsatile roller pump (*Holley et al. 1982*). The patient blood is diluted to a haematocrit in the mid-20s by the pump priming solution, temperature is cooled down to 27°C to decrease the metabolic requirements, and perfusion flow and pressure are less than normal leading to altered regional blood flow (*Holley et al. 1982*). Thus cardiopulmonary bypass (CPB) involves a number of alterations in the normal physiology

such as hemodilution, altered protein concentration, hypothermia and diminished hepatic and renal function.

1.8.1 Cardiopulmonary Bypass and Pharmacokinetics of Drugs

The profound changes during CPB procedure have the potentials for altering the pharmacokinetics and pharmacodynamics of administered drugs. The effect of CPB on the pharmacokinetics of drugs has been reviewed by Holley *et al.* (*1982*) and Buylaert *et al.* (*1989*). Blood levels of some drugs such as gallamine (*Shanks et al. 1983*), alcuranium (*Walker et al. 1983*), tubocurarine (*Walker et al. 1984*) and cephalothin (*Miller et al. 1979*) were increased during CPB, while decreases were observed for lorazepam (*Aaltonen et al. 1982, Boscoe et al. 1984*), midazolam (*Chantey et al. 1985*), pancuronium (*d'Hollander et al. 1983*), propranolol (*Plancetka et al. 1981*), digitoxin (*Storstein et al. 1979*), cefazolin (*Miller et al. 1980*) and fentanyl (*Koren et al. 1984a*). Plasma nifedipine level was decreased during and after bypass but the decrease was not due to the CPB itself but rather to the normal metabolism and elimination of the drug (*Katz et al. 1990*). Similarly, lidocaine kinetics were unchanged 15 min after CPB (*Holley et al 1984*). This shows that the effects of complex interactions during CPB vary from drug to drug.

The increase in the volume of distribution due to the pump volume itself and binding to its constituent parts, including the oxygenator, is potentially significant (*Hynynen 1987, Koren et al. 1984b, Rosen et al. 1988, Skacel et al. 1986*). In a study with nitroglycerin, Booth *et al.* (*1991*) have shown that at steady state the circulating

drug was decreased by 21%, 47% and 67% with the Maxima membrane oxygenator, Cobe membrane oxygenator and Bentley bubble oxygenator, respectively. Similar sequestration of fentanyl but not alfentanil by oxygenators has been reported (*Hynynen 1987, Koren et al 1984b, Skacel et al. 1986*). It is interesting to note that some basic drugs such as fentanyl and propranolol accumulate in the lung during CPB, and with the resumption of blood flow to the lung the accumulated drug is washed out; this results in an elevated plasma level after cessation of CPB (*Bently et al. 1983, Plachetka et al. 1981*).

Altered hepatic drug metabolism is expected under conditions of hypothermic CPB. Hypothermia has been shown to reduce the metabolism of drugs such as propranolol, verapamil and fentanyl (*Koren et al. 1987, McAllister and Tan 1980*). Koska *et al.* (*1981*) reported that fentanyl elimination half-life after CPB was longer and they suggested that decreased (30%) liver blood flow was responsible for this effect. Pancuronium is shown to be subject to considerable tubular reabsorption and its renal excretion was found to be increased by hypothermia during CPB (*Wierda et al. 1990*). One of the limitation of pharmacokinetic analysis during and after CPB is that there are continuous physiological changes that render pharmacokinetic evaluation difficult.

In assessing the effect of CPB on pharmacokinetics it is important to determine the unbound drug concentration since it is the active moiety. Kumar *et al.* (*1988*) found that plasma alfentanil levels decreased with the onset of CPB but the unbound concentration was essentially unchanged. Plasma albumin and AAG concentrations fell to 50% of pre-CPB levels with a strong correlation between the bound/unbound drug and

plasma AAG concentration. No changes in unbound drug have been shown for methohexital and thiopental (*Bjorksten et al. 1988*). Based on these studies it appears that total drug concentration is decreased at the onset of CPB but the unbound drug concentration is not affected. The decrease in plasma protein binding secondary to decreased plasma proteins increases the unbound fraction of drug. This explains why the unbound drug concentration is not changed although the total drug concentration is decreased. Results of Kumar *et al.* (*1988*) suggest that hemodilution could not account for the pronounced decrease in plasma proteins. It is possible that some of the binding protein is adsorbed onto parts of the CPB device during surgery. Another factor, increased free fatty acids level induced by both heparin and by the onset of CPB, has been attributed to the increased unbound fraction of cefatriaxone and propranolol during CPB (*Jungbluth et al. 1989, Wood et al. 1979*).

1.8.2 Use of Diltiazem in Patients Undergoing Cardiopulmonary Bypass

The incidence of myocardial ischemia in the immediate postoperative period after coronary artery bypass grafting is essentially the same as that during the preoperative period, 40% and 42% respectively, despite the fact of coronary revascularization (*Knight, 1988*). There are reports of coronary artery vasospasm during general anaesthesia prior to CPB (*Buffington and Ivey 1981*), on termination of CPB subsequent to $CaCl_2$ injection (*Boulanger et al. 1984*), in the immediate perioperative period (*Buxton et al. 1981*) and during the early postoperative period (*Klaus et al. 1981*). Also myocardial ischemia results in increased membrane permeability to calcium and, particularly during

reperfusion, intracellular calcium will rise causing further myocardial damage (*Braunwald and Kloner 1985, Malendez et al. 1988*).

Diltiazem is effective in the prevention of coronary artery vasospasm during coronary artery bypass surgery (*Flaim and Zelis 1981a, Fuse et al. 1988*). It has a protective role during myocardial ischemia, reduces arrhythmias associated with ischemia and may protect the ischemic myocardium from calcium induced damage during reperfusion (*Clozel et al. 1983, Flaim and Zelis 1981b, Godet et al. 1987, Krukenkamp et al. 1986*). These observations suggest that diltiazem may be useful as an adjunct for the pharmacological support of the bypassed heart during open heart surgery. It appears that a therapeutic level of free diltiazem is desirable prior to CPB, during CPB and immediately after CPB.

1.9 Time Dependent Kinetics of Diltiazem

Several drugs alter their own clearance during repeated administration and this phenomenon is often referred to as time-dependent kinetics. There is a significant accumulation of diltiazem during chronic oral dosing and the AUC, C_{max}, steady state diltiazem concentration and bioavailability increase more than what can be predicted from a single dose study (*Hoglund and Nilsson 1989a, Hung et al. 1988, Smith et al. 1983*). Single dose studies in healthy volunteers have shown that the AUC is linear up to 120 mg of oral diltiazem (*Kinney et al. 1981, Smith et al. 1983*) and also the peak plasma level is linear in a dose range of 60 to 210 mg of oral diltiazem (*Morselli et al. 1979*). Although the increased bioavailabilty and accumulation after multiple dosing have been

attributed entirely by some authors to the saturation of first-pass metabolism, single dose studies do not support the saturation of first-pass metabolism.

Like diltiazem, lidocaine, verapamil and diphenhydramine analogs also show time-dependent pharmacokinetics (*Bauer et al. 1982, Eichelbaum and Somogyi 1984, Labout et al. 1982, Lennard et al. 1983, Ochs et al. 1980, Meredith et al. 1985, Schwartz et al. 1985*). A common property of all these drugs and diltiazem is that they all have a secondary or tertiary amino group in their structure and they are mainly metabolised in the liver by *N*-dealkylation (*Bast and Noordhoek 1982, Bast et al. 1984, Keenaghan and Boyes 1972, McIlhenny 1971, Sugihara et al. 1984*). It has been shown that diphenhydramine and its analogs have the ability to inactivate some P450 isozymes by the formation of stable metabolic intermediate complexes. The result is time-dependent kinetics (*Bast et al. 1984, Bast et al. 1990, Labout et al. 1982*). Inactivation of P450 by these secondary and tertiary amines requires the prior *N*-oxidation of *N*-alkyl groups. Metabolic intermediate complexes of P450 have been observed with a variety of amphetamines, SKF 525-A and derivatives of methylenedioxybenzene (*Gray and Tam 1991*). The time-dependent kinetics of lidocaine are related to the inactivation of the *N*-dealkylating enzyme system (*Saville et al. 1989, Tam et al. 1987*). From those observations, it appears that a similar mechanism is responsible, at least partially, for the time-dependent kinetics of diltiazem.

The clearance of diltiazem is hepatic blood flow dependent (*Piepho et al. 1982*). Since diltiazem is a vasodilator, any change in hepatic blood flow rate will change the clearance of the drug. It has been reported that diltiazem does not change liver blood

flow (*Bauer et al. 1986*), thus eliminating the possibility of a blood flow effect on hepatic clearance of diltiazem. Similarly, a change in free fraction of diltiazem during multiple dosing cannot explain the time-dependent effect since diltiazem plasma protein binding is independent of concentration in the therapeutic range (*Belpaire and Bogaert 1990, Pieper 1984a*).

1.10 Isolated Perfused Rat Liver Model

The rat has been used to study the metabolism and pharmacokinetics of diltiazem (*Meshi et al. 1971, Nakamura et al. 1987, Yeung et al. 1990*). The reason for choosing this animal model was that the metabolic pattern of diltiazem in rats is qualitatively similar to that found in man (*Meshi et al. 1971*).

Isolated perfused rat liver (IPRL) is a convenient preparation for studying the kinetics and dynamics of transport, binding and metabolism of drugs. In the structural and functional hierarchy of experimental models, it is the closest to the *in vivo* situation, as compared to the isolated cell and microsomal preparations. In contrast with isolated hepatocytes and isolated organelles, hepatic architecture, cell polarity and bile flow are preserved in IPRL (*Gores et al. 1986*).

The use of a 'single-pass' system avoids the complications arising from the recirculation of test substance and allows the researcher to study the individual parameters at steady state. 'Single-pass' IPRL has been used to determine the effect of enzyme inactivation on drug pharmacokinetics and drug-drug interactions (*Bruck et al. 1990, Tam et al. 1987*).

1.11 Hypotheses

1. The pharmacokinetics of diltiazem are changed during coronary artery bypass surgery.
2. Supplemental diltiazem is necessary to maintain the therapeutic level of diltiazem during and immediately after coronary artery bypass graft operation.
3. Inactivation of P450 enzymes contributes to the time-dependent pharmacokinetics of diltiazem.
4. Reversible tissue binding contributes to the time-dependent pharmacokinetics of diltiazem.
5. Inactivation of P450 enzymes by pretreatment with diltiazem and other tertiary amines such as lidocaine, diphenhydramine and verapamil will effect the kinetics of diltiazem.
6. Coadministration of lidocaine and diphenhydramine with diltiazem will cause drug interactions due to inhibition of metabolic pathways.

1.12 Objectives

1. To develop a simple, sensitive and specific HPLC method to determine plasma concentrations of diltiazem and six of its metabolites.
2. To determine pharmacokinetic parameters of diltiazem in patients who needed coronary artery bypass grafting, both before and on the day of surgery.
3. To determine the mechanisms of time-dependent pharmacokinetics of diltiazem in the rat.
4. To evaluate the effect of pretreatment of diltiazem, lidocaine, diphenhydramine and verapamil on the kinetics of diltiazem.
5. To determine the mechanisms of interaction when diltiazem is coadministered with either lidocaine or diphenhydramine.

1.13 Figures

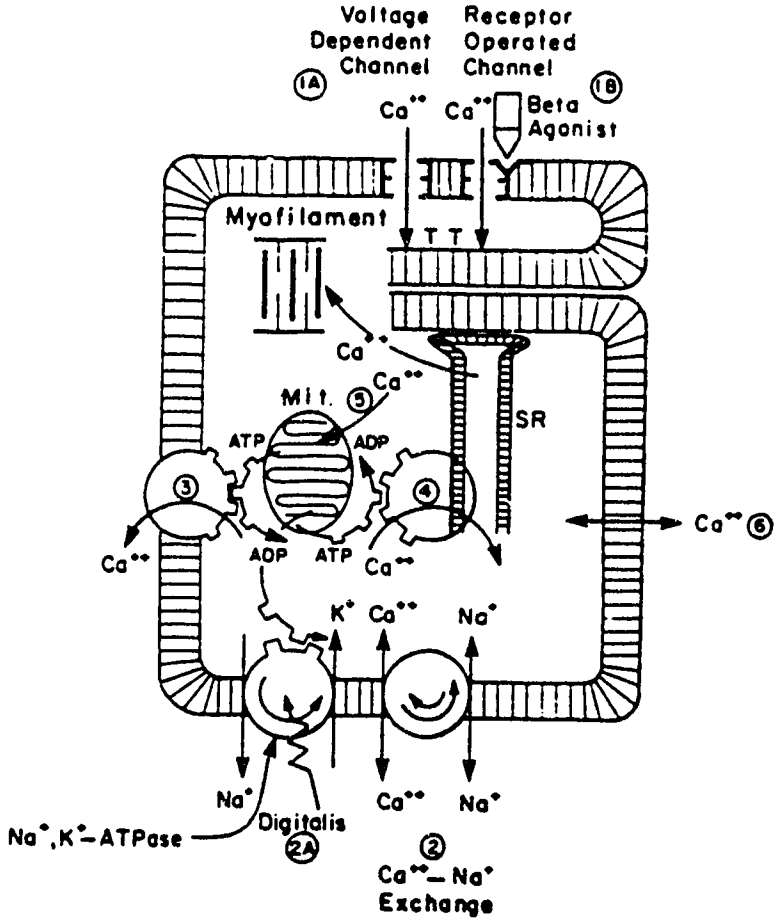

Figure 1.1. The regulation of Ca^{2+} in myocardium. TT denotes transverse tubule, SR sarcoplasmic reticulum and Mit. mitochondrion. Numbers in circles denote the mechanisms affecting intracellular Ca^{2+} (Braunwald 1982).

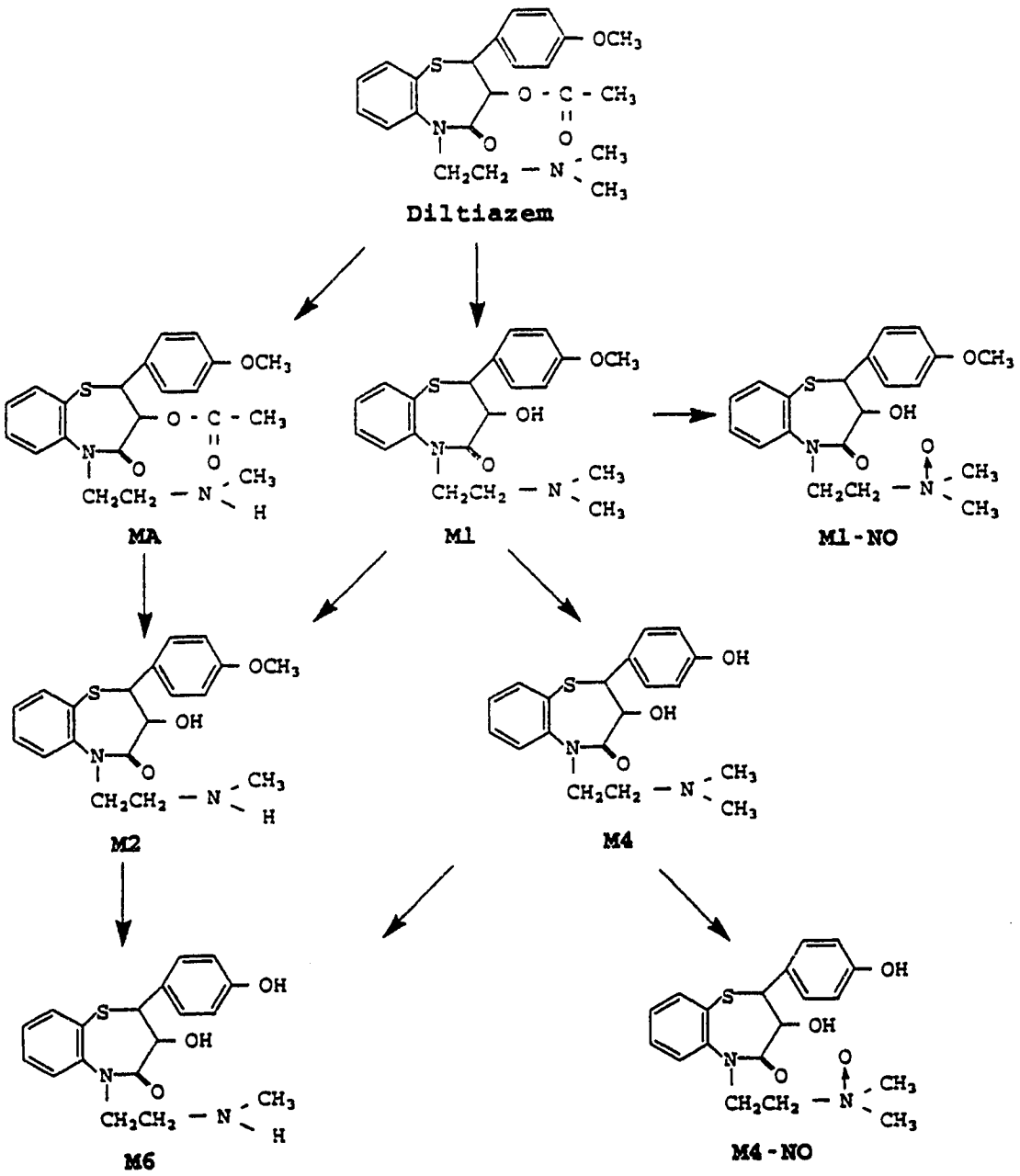

Figure 1.2 Proposed metabolic pathways of diltiazem in the human.

1.14 References

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2. A SIMPLE AND SENSITIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF DILTIAZEM AND SIX OF ITS METABOLITES IN HUMAN PLASMA[1]

2.1 Introduction

Diltiazem, a CEB, is effective in the treatment of angina pectoris (*Buckley et al. 1990, Zelis 1982*) hypertension (*Inouye et al. 1984, Maeda et al. 1981*) and cardiac arrhythmias (*Akhtar et al. 1989, Rozanaski et al. 1982*). Diltiazem undergoes extensive presystemic elimination and is mainly metabolised by the liver (*Buckley et al. 1990, Meshi et al. 1971*). The major phase I metabolic pathways are *N*-demethylation and deacetylation in the human (*Buckley et al. 1990, Hoglund and Nilsson 1989a, Yeung et al. 1990*). The primary product of *N*-demethylation and deacetylation are MA and M1, respectively and they undergo further metabolism to M2, M4 and M6. The formation of M1-NO or *O*-demethyldeacetyldiltiazem *N*-oxide (M4-NO) by *N*-oxidation also occur (*Caille et al. 1989, Yeung et al. 1990*). The metabolic pathways of diltiazem are shown in chapter 1, figure 1.2. The metabolites of diltiazem share the pharmacological properties of diltiazem (*Kiyomoto et al. 1983, Yabana et al. 1985*). Metabolites MA and M1 have coronary vasodilating potencies amounting to 50% (*Rovei et al. 1980*) and 20% (*Sugihara et al. 1984*) of that of diltiazem. Metabolites M1 and M4 are more potent than diltiazem in inhibiting adenosine uptake in erythrocyte and platelet aggregation

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(*Kiyomoto et al. 1983, Yeung et al. 1991*). Thus, it is important to measure the metabolites of diltiazem.

Several HPLC (*Boulieu et al. 1990, Goebel et al. 1985, Hoglund and Nilsson 1987, Johnson and Pieper 1987, Leneveu et al. 1991*) and GC (*Belanger et al. 1987, Clozel et al. 1984*) assays for diltiazem and its metabolites in plasma have been published. Unfortunately these methods cannot quantify all the known metabolites of diltiazem and they also lack the sensitivity and specificity required to study their *in vivo* kinetics. In order to study the pharmacokinetics and drug interactions of diltiazem in humans and animals, a simple, sensitive and specific assay capable of measuring diltiazem and all its known metabolites is needed. Yeung *et al.* (*1989*) recently developed an HPLC assay to determine diltiazem and six of its metabolites in the plasma of humans but this assay is tedious and a chromatogram of the blank plasma is not completely free of endogenous interfering peaks. Also the metabolites are not baseline resolved. Using 2 ml of plasma, the reported sensitivity of the method is 10 ng/ml with large assay variation (up to 60%). We have developed a simple, specific and sensitive HPLC assay that permits quantification of diltiazem and six of its metabolites. This method was used to study pharmacokinetics of diltiazem and its metabolites in patients receiving diltiazem chronically.

2.2 Experimental

2.2.1 Chemicals

The hydrochloride salt of diltiazem and its metabolites M1, M2, M4, M6, M1-NO and the fumarate salt of MA were kindly donated by Nordic Merrel Dow Research (Laval, Quebec, Canada). Internal standard (I.S.) benzylamphetamine hydrochloride was synthesized in our laboratory. Ethyl acetate and acetonitrile were HPLC grade solvents (BDH, Toronto, Canada). All other chemicals were of analytical grade and were obtained from BDH, Toronto, Canada.

2.2.2 Instruments

The HPLC instrumentation consisted of a Model 710B automatic injector, a Model 501 pump and a Model 441 UV detector (all from Waters, Mississauga, Canada). The detector was set at 214 nm. Chromatographic separation was achieved on a Waters 4μm, C_{18} reversed phase Novapak cartridge column (10 cm x 8 mm I.D.). The mobile phase was comprised of an aqueous solution of 0.045% H_3PO_4 and 0.1% triethylamine (65 parts) and acetonitrile (35 parts) and was pumped at a flow rate of 2 ml/min.

2.2.3 Standard Solutions

Stock solutions of diltiazem and its metabolites were prepared separately in distilled water to a final concentration of 100 μg/ml base equivalent. The internal standard benzylamphetamine concentration was 10 μg/ml. Drug free human plasma

samples (supplied by Red Cross) were spiked with solutions of diltiazem and its metabolites. Serial dilution with plasma gave concentrations of 5, 10, 20, 40, 100, 200, 500 ng/ml of diltiazem and the metabolites. All labware was made of glass and the caps of tubes were teflon lined.

2.2.4 Sample Preparation

At the time of analysis, plasma samples were equilibrated to room temperature. To 1 ml of plasma containing diltiazem and its metabolites in a glass test tube was added 50 μl of I.S. solution and excess $KHCO_3$ (~ 1 g). When effervescence stopped, the mixture was vortexed with 4 ml of ethyl acetate for 15 min on a vortex shaker (IKA-VIBRAX-VXR, Terrochem, setting at 1200). After centrifugation (1000 *g*) for 10 min, the aqueous layer was frozen in a dry ice/acetone bath. The organic layer was decanted into another test tube and the aqueous layer was discarded. To the separated organic layer, 0.2 ml of 0.01 M HCl was added. The mixture was vortexed for 10 min and centrifuged for 10 min at 1000 *g*. The aqueous layer was again frozen (dry ice/acetone bath) then separated and dried under nitrogen. The dried residue was dissolved in 0.2 ml of 0.002 M HCl. Aliquots of 0.10 to 0.15 ml were injected onto the HPLC system.

2.2.5 Assay Validation

To establish the accuracy and precision of the assay, a validation study was done using quality control (QC) samples prepared by an authorized person in the laboratory. The concentration of these QC samples covered the range of calibration curve. Inter-day

variations of QC samples were evaluated on three different days. Each sample was analyzed in duplicate on all three occasions. For the intra-day variation study, four additional samples at each of the concentrations were analyzed on the third experimental day.

2.2.6 Patient Study

The applicability of the assay was elucidated by measuring diltiazem and its six metabolites in three patients scheduled for coronary artery bypass grafting. Informed consent was obtained and the patients were examined for liver and renal diseases. They were receiving oral diltiazem (Cardizem^{R} tablets, Nordic Research Inc., Laval, Quebec, Canada), 60 mg q.i.d. for at least three months. On the day of study, an indwelling catheter was inserted into an antecubital vein prior to the oral dose of diltiazem. Blood samples were collected at 0, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 h and then at every hour up to 8 h. Subsequent dosing of diltiazem was withheld until after the 8th hour sample. The eight hour sampling period was to characterize properly the half-life ($t_{1/2}$) of diltiazem and its metabolites. Each blood sample was immediately centrifuged (1500 *g*) and plasma harvested and stored at -20° C until analysis. Samples were analyzed within eight weeks after collection.

2.2.7 Data Analysis

Standard curves (5-500 ng/ml for diltiazem and its metabolites) were constructed by plotting the peak area ratio of diltiazem or its metabolites to the internal standard *vs*

spiked plasma concentrations. Each spiked plasma standard was analyzed in triplicate. Linear regression analysis was used to determine the best fit line through each set of standards.

Plasma concentration *vs* time data were analyzed using the computer program LAGRAN (*Rocci and Jusko 1983*). Terminal half-life ($t_{½}$) was calculated from the terminal disposition rate constant ß, using the relationship $t_{½} = 0.693/ß$. Terminal disposition rate constant ß was determined by using linear regression of the terminal log linear portion of the concentration-time profiles. Maximum concentration (C_{max}) and time to achieve this value (T_{max}) were obtained directly from the data. Data are presented as mean ± SD.

2.3 Results

Chromatograms obtained from a blank plasma sample and a plasma sample spiked with diltiazem (200 ng/ml) and its metabolites (100 ng/ml) are shown in Figures 2.1A and 2.1B. These chromatograms indicate that the drug and metabolite peaks are well resolved and endogenous interference was absent.

The method was applied to measure diltiazem and its metabolites in three patients. Figure 2.1C is a chromatogram of a plasma sample collected from one patient. All six metabolites were found in this patient. In addition to the six peaks of known composition, two peaks at 4.7 and 5.7 min which contained unknown components that did not interfere with the analysis, were also detected in all three patients we studied.

These peaks may represent unknown diltiazem metabolites or may be contributed by concomitantly administered drugs.

Calibration curves prepared from standard solutions were linear ($r^2 > 0.99$) in the concentration ranges studied (5-500 ng/ml for both diltiazem and its metabolites). Results of the validation study are presented in Table 2.1. The coefficient of variation, an indicator of the precision, was less than 10% for diltiazem and metabolites for both inter-day and intra-day assay. The estimated concentration expressed as the percentage of the spiked concentration revealed the accuracy of the method which ranged from 93-105%. Using our extraction procedure, recoveries of diltiazem and its metabolites were more than 75% with the exception of M1-NO, which was 41.9 ± 5.95% and 27.8 ± 2.0% at concentrations 200 and 10 ng/ml respectively. A similar finding for M1-NO (~30%) was reported by Yeung *et al.* (*1989*) who attributed the poor recovery of M1-NO to adsorption onto glass or other surfaces and high aqueous solubility. We found that a mixture of methylene chloride and ethyl acetate in the ratio of 1:3 or 1:4 increased the extraction efficiency of M1-NO, M6 and M4 slightly but decreased the extraction efficiency of M1, MA and diltiazem and thus the mixture of methylene chloride and ethyl acetate has no advantage over ethyl acetate alone.

The plasma concentration *vs* time profiles of diltiazem and its metabolites in one patient are provided in Figure 2.2 and the pharmacokinetic parameters are presented in Table 2.2. The pharmacokinetic data presented here are consistent with literature values (*Hoglund and Nilsson 1989b, Montamat et al. 1987, Smith et al. 1983, Yeung et al. 1990*). MA, M1 and M2 were the major metabolites present in the patients examined

by us. Their levels were less than that of diltiazem. M1-NO was not detectable in patient FM. The calculated oral clearance (D_o/AUC_{0-6}) of 70.4 L/h (1.17 L/min) in those patients was within the reported limits (*Smith et al. 1983, Yeung et al. 1990*).

2.4 Discussion

When compared to the analytical procedure described by Yeung *et al.* (*1989*) which is the only reported method that permits quantification of diltiazem and its six metabolites, the method now described is relatively simple and rapid. The method described by Yeung *et al.* (*1989*) requires two step extraction followed by drying of 10 ml organic phase and after reconstitution, washing twice with methyl *tert*-butyl ether and hexane. Sample treatment in our assay involves only one extraction and a back extraction. The calibration curve for diltiazem can be extended up to 1000 ng/ml and a linear response is still obtained. Using the extraction procedure reported here, the quantifiable limit in plasma is 5 ng/ml for diltiazem and its metabolites and the detection limit for the metabolites is <2 ng/ml. A plasma volume ranging from 0.5 to 1.0 ml is found to be adequate in quantifying low diltiazem and metabolite concentration using our method. The assay procedure described by Yeung *et al.* (*1989*) requires a larger volume of plasma, is less sensitive and has a larger inter-assay variability.

The addition of $KHCO_3$ was to render the plasma alkaline (pH ~ 8.3-8.5) for better extraction as a lower pH resulted in poor recoveries and a higher pH would cause hydrolysis of diltiazem and MA (*Abernethy et al. 1985, Hoglund and Nilsson 1987, Weins et al. 1984*). Optimal extraction with no degradation is achieved at pH <8.5

(*Abernethy et al. 1985, Weins et al. 1984*). Hoglund and Nilsson (*1987*) found that saturating the plasma with sodium chloride increased the extraction efficiency of MA, M1, M2 and M4 with a hexane and 2-propanol (95:5) mixture. The use of $KHCO_3$ in our method provides a plasma pH optimum for extraction and may have increased the extraction efficiency of the ethyl acetate. Under this extraction condition diltiazem and its metabolites were stable. Drying the back-extracted aqueous phase gives a clearer chromatogram.

Several compounds were investigated for use as internal standard. Since diltiazem and its metabolites differ widely in their physicochemical properties, the selection of an ideal internal standard is difficult. Benzylamphetamine, which is not used as a drug, was chosen as internal standard because of its clear separation from diltiazem and its metabolites, its appropriate retention time (earlier than diltiazem, 14.3 min), symmetrical peak shape and good extraction recovery. The likelihood of analytical interferences from other drugs that could be administered during diltiazem therapy was examined. Lidocaine and its metabolites *N*-ethylglycyl-2,6-xylidide (MEGX) and glycyl-2,6-xylidide (GX), diphenhydramine, metoprolol, bupivacaine, all of which may be present in patient plasma along with diltiazem and its metabolites, did not interfere with the assay.

The C_{18} Novapak cartridge column could be utilized for at least six months or for the analysis of more than 500 samples without any deterioration in its performance.

MA and M1 are often identified as the major metabolites of diltiazem but M2 should also be considered as a major metabolite of diltiazem. Its C_{max} and AUC values were comparable to those of MA and M1 in these three patients studied. Yeung *et al.*

(1989), in a single 90 mg oral diltiazem study, made a similar observation. Also they could not detect M1-NO in two out of four volunteers. M1-NO was found in two patients in our study. The other two metabolites M6 and M4 whose pharmacokinetic data have not been reported in any other studies, were also detected and quantified in these patients. It is possible that these metabolites have accumulated after chronic oral diltiazem and the sensitivity of the present assay has allowed their quantification.

The HPLC method described here displayed adequate performance with respect to precision, accuracy and specificity. It is linear and reproducible within the plasma concentration range observed in patients. The method is rapid, sensitive and is applicable for the determination of the pharmacokinetics of diltiazem in man. Currently this method has been applied to pharmacokinetic studies in patients undergoing coronary artery bypass graft operation and it is also used in our laboratory to study the time-dependent pharmacokinetics and drug interactions of diltiazem in the dog.

2.5 Tables

Table 2.1 Accuracy and precision of the assay for diltiazem and its metabolites (n=6).

Species	Conc added (ng/ml)	Intra-day conc. found (ng/ml)	Inter-day conc. found (ng/ml)
Diltiazem	500	503.4 ± 20.8	505.7 ± 19.6
	200	194.6 ± 6.31	195.8 ± 8.81
	10	10.03 ± 0.55	10.10 ± 0.84
MA	250	233.3 ± 4.61	237.9 ± 9.30
	100	103.2 ± 4.65	97.97 ± 5.18
	5	4.92 ± 0.28	5.18 ± 0.39
M1	250	251.2 ± 6.71	246.0 ± 10.5
	100	95.10 ± 3.90	100.0 ± 4.23
	5	4.69 ± 0.46	5.00 ± 0.52
M2	250	246.6 ± 6.50	242.6 ± 7.78
	100	96.22 ± 4.02	99.70 ± 3.31
	5	4.98 ± 0.32	4.96 ± 0.26
M4	250	251.4 ± 13.1	244.1 ± 15.0
	100	98.82 ± 5.61	100.4 ± 2.73
	5	5.08 ± 0.72	5.24 ± 0.28
M6	250	246.3 ± 2.93	245.4 ± 11.6
	100	104.1 ± 5.55	100.5 ± 4.88
	5	5.15 ± 0.38	4.97 ± 0.27
M1-NO	250	256.4 ± 11.5	247.1 ± 12.7
	100	93.57 ± 6.49	99.68 ± 4.74
	5	4.98 ± 0.46	4.93 ± 0.52

Table 2.2 Pharmacokinetic parameters of diltiazem and its metabolites in three patients receiving 60 mg of diltiazem q.i.d.

Species	C_{max}, ng/ml	T_{max}, h	$t_{½}$, h	AUC_{0-6},ng/ml.h
Diltiazem	177.5 ± 22.3	2.7 ± 0.6	4.2 ± 2.6	852.1 ± 123.0
MA	32.9 ± 5.2	3.7 ± 1.5	5.7 ± 3.0	155.3 ± 29.0
M1	32.5 ± 9.4	3.0 ± 0.0	6.1 ± 1.7	135.2 ± 25.8
M2	29.8 ± 13.3	3.3 ± 0.6	3.7 ± 2.4	142.8 ± 52.3
M4	9.9 ± 5.1	3.3 ± 1.5	3.8 ± 1.5	45.9 ± 21.6
M6	14.7 ± 3.9	4.0 ± 1.73	3.0 ± 2.1	61.6 ± 6.9
M1-NO*	20.6	1.3	5.3	93.5

* M1-NO was detectable in the plasma of two patients.

2.6 Figures

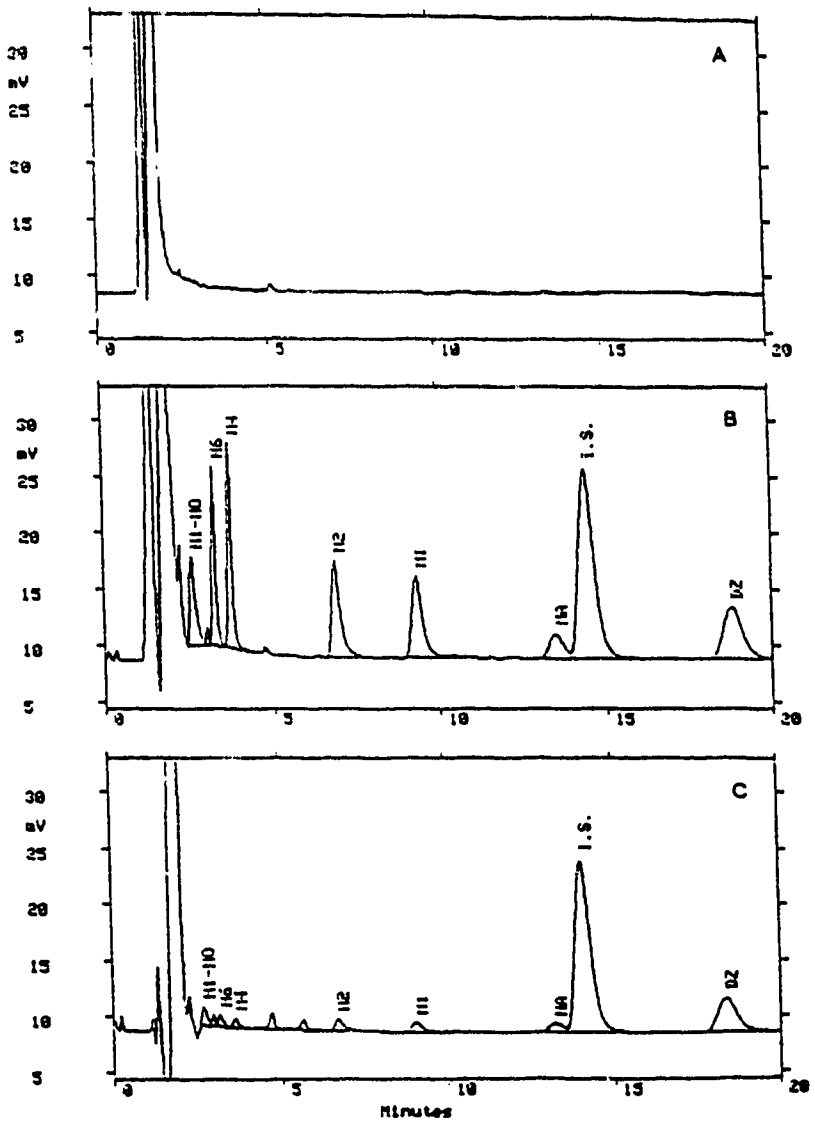

Figure 2.1 Chromatogram of drug free plasma (A), plasma spiked with 200 ng/ml of diltiazem (DZ) and 100 ng/ml of metabolites (B) and a plasma sample from a patient receiving 60 mg DZ q.i.d. (C). The signals were measured in mV.

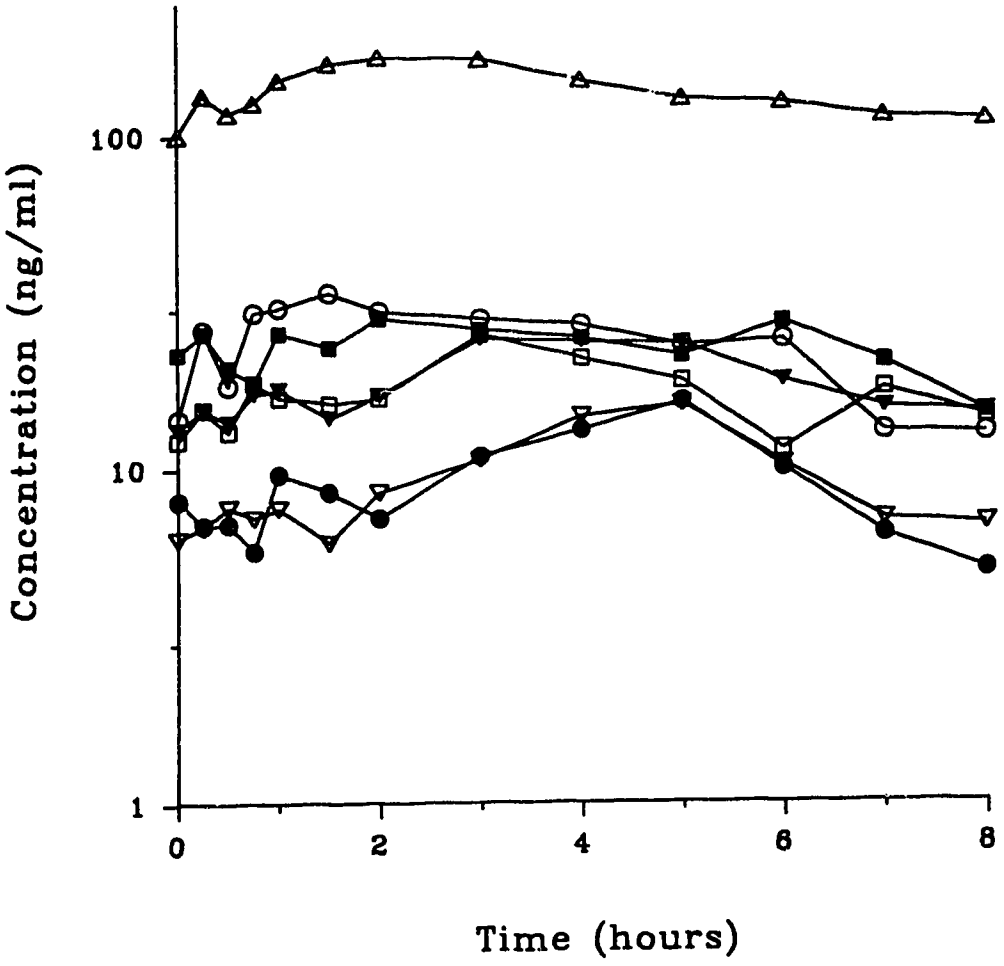

Figure 2.2 Plasma concentration time profile of diltiazem (DZ) and its metabolites in a patient receiving 60 mg q.i.d. DZ (○ M1-NO, ● M6, ▽ M4, ▼ M2, □ M1, ■ MA, △ DZ).

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3. PHARMACOKINETICS OF DILTIAZEM IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING[2]

3.1 Introduction

Diltiazem has been demonstrated to be beneficial in the management of ischemia (*Crawford 1989*), hypertension (*Inouye et al. 1984*) and supraventricular arrhythmias (*Akhtar et al. 1989*). Patients presenting for coronary artery bypass surgery, who have been managed medically, are usually under therapy with a CEB, such as diltiazem. In these patients, the standard paradigm of preoperative medication is to administer the CEB orally prior to induction of anaesthesia. During the intraoperative and immediate postoperative period the initial oral dose of the CEB is not supplemented. Perioperative events associated with coronary artery bypass surgery such as coronary artery vasospasm (*Buffington and Ivey 1981, Buxton et al. 1981*) and reperfusion injury (*Braunwald and Kloner 1985*) can be attenuated by the presence of therapeutic concentrations of CEB's (*Clozel et al. 1993, Flaim and Zelis 1981*). It is not known whether the normal practice provides an adequate concentration of CEB intraoperatively.

There is little information on the fate of CEB's given orally to patients undergoing cardiac surgery (*Katz et al. 1990*) and none on the effect of CPB on the relationship between unbound and bound drug. CPB is associated with profound changes in the distribution of organ blood flow, hemodilution, altered protein concentrations, and

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[2] A version of this chapter has been published. Finegan BA, Hussain MD, Tam YK. *Ther Drug Monit* 1992;14:485-492.

hypothermia and significantly alters drug distribution and metabolism (*Buylaert et al. 1989*). As diltiazem is extensively metabolized and highly plasma protein bound (*Buckley et al. 1990*), the effects of CPB on the kinetics of diltiazem are of particular interest.

The objective of this study was to characterize the pharmacokinetics of preoperative oral diltiazem in patients undergoing cardiac bypass surgery in order to identify if therapeutic levels of unbound diltiazem were present during and immediately following CPB. Patients were studied on the day prior to and the day of surgery so that the effect of CPB on the pharmacokinetics of diltiazem could be clearly evaluated.

3.2 Patients and Methods

The study was approved by the Human Ethics Committee of the University of Alberta. Patients, who were scheduled for elective coronary artery bypass grafting and were treated with oral diltiazem for at least three months, were eligible for inclusion in the study. Patients with a history of noncompliance with diltiazem therapy, gastrointestinal surgery, liver or renal disease and patients receiving concomitant drugs known to effect enzyme activity were excluded from the study. Furthermore, only patients scheduled for the first operation of the day (0600 h) were included and if a delay in the schedule occurred the patient was excluded from further participation in the study. Twenty seven patients were enroled in the study after informed consent was obtained. Ten patients completed the study (nine males, one female; mean age 60.4 $\pm$ 6.0 yr; mean weight 83.9 $\pm$ 9.5 kg; mean height 170.8 $\pm$ 7.1 cm), the remainder of the patients

were excluded for the reasons of cancellation or delay in surgery. Six subjects were taking diltiazem 60 mg q.i.d and four 90 mg diltiazem q.i.d.

Each patient was studied on the day prior to surgery and on the day of surgery. On the day prior to surgery at 0550 h an indwelling catheter was inserted into a vein in the forearm and a baseline blood sample drawn. The patients received an oral dose of diltiazem (60 mg or 90 mg) which was given at 0600 h and blood samples (five ml) were collected at 15 min intervals for the first hour, 30 min intervals for the second hour and hourly thereafter until eight hours had elapsed. The subsequent dose of diltiazem was withheld until sampling was complete. The rationale for an eight hour sampling schedule was to properly characterize the half-life ($t_{\frac{1}{2}}$) of diltiazem and its deacetyl [illegible] *N*-demethyl (MA) metabolites. Patients were allowed to carry out their routine [illegible]ive activities including normal fluid and food intake.

On the day of surgery the method of blood sampling, the timing of diltiazem administration and the dose of diltiazem administered was identical to that of the preoperative day except that samples were obtained from an indwelling catheter in the radial artery. This was to ensure a patent catheter for sampling during the operative period. Samples were collected at 15 min intervals for the first hour, 30 min intervals for the remaining seven hours except during CPB when sampling was at 15 min intervals. Additional samples were obtained postoperatively at study hours 12 and 24.

Patients were premedicated with diazepam (0.1 $mg.kg^{-1}$) orally and morphine sulphate (0.1 $mg.kg^{-1}$) parenterally and anaesthesia was induced and maintained with

midazolam and fentanyl. Induction of anaesthesia and onset of CPB occurred approximately 90 and 240 min into the sampling period respectively.

Blood samples were drawn into glass tubes. After coagulation, the samples were centrifuged at 1500 *g* for ten minutes, the serum harvested and stored at -20°C for later analysis. Diltiazem and its metabolites, M1 and MA, in serum were quantitated using our newly-developed, simple and sensitive HPLC method (Chapter 1). The inter-day and intra-day coefficient of variation for the drug and its metabolites were less than 10%, using this assay.

An equilibrium dialysis method was used to evaluate serum protein binding of diltiazem. A 0.5 ml sample of patient serum, spiked with 125 nCi of ^{3}H-diltiazem (NEN, Mississauga, Ontario, Canada), was dialysed against an isotonic Sorensen phosphate buffer (pH = 7.4) at 37°C for 3 h, using a Spectrum equilibrium dialyser (Spectrum, Los Angeles, CA, USA). The serum and the buffer compartment was separated by a dialysis membrane (Spectrum) with molecular weight cutoff between 12,000 to 14,000. Three hundred μl of serum and buffer were individually removed and added to 3.5 ml of ScintiVerse™ (Fisher, Nepean, Ontario, Canada). Radioactivity of each sample was measured using a Beckman liquid scintillation counter (model LS 9000, Beckman Instruments, Inc., Palo Alto, CA, USA). Quench correction was achieved using an external standard ratio method. Unbound fraction (FF) was calculated by dividing radioactivity (dpm) on the buffer side by the plasma side. Unbound drug concentration was calculated by multiplying plasma diltiazem concentration by the FF.

Pharmacokinetic parameters, AUC and $t_{½}$ were calculated from the plasma concentration *vs* time data for diltiazem and its metabolites, during a dosing interval, using the computer program LAGRAN (*Rocci and Jusko 1983*). Maximum concentration (C_{max}) and the time to achieve this value (T_{max}) were directly recorded from the data. Oral clearance (Cl_o) of total and unbound diltiazem were calculated using the following equation:

$$Cl_o = \frac{Cl}{F} = \frac{D_o}{AUC_{0\text{-}6}}$$

where, Cl is total body clearance, F is bioavailability, D_o is oral dose and $AUC_{0\text{-}6}$ is the area under plasma concentration *vs* time curve for a 6 h dosage interval.

The unpaired Student's t test was used to compare kinetic data between the two doses (C_{max}, T_{max}, $t_{½}$, AUC, Cl/F and FF). The paired Student's t test was used to compare kinetic data obtained from the day before and the day of surgery (C_{max}, T_{max}, AUC and FF). One way analysis of variance (ANOVA) was used to evaluate plasma concentration of drug and metabolite data immediately before, during, immediately after and 1 hour after the CPB procedure. Tukeys test was used for multiple comparison of means, if a difference was detected. The level of significance, P, was set at 0.05. Data are presented as mean $\pm$ SD.

3.3 Results

As the onset and duration of CPB differ slightly from patient to patient and the timing of blood samples were also different during the surgery, it is not feasible to present mean plasma concentration profiles. Therefore, plasma concentration profiles of total (TDZ), unbound diltiazem (FDZ) and its two major metabolites, MA and M1 of two representative patients: one from each dosage group are shown (Figure 3.1). Consistent with the observation of Smith *et al.* (*1983*), diltiazem showed multiple plasma peaks after dosing, suggesting potential enterohepatic recycling. There was an apparent lag time of absorption ranging from 15-30 minutes in all patients and at all occasions. Pharmacokinetic parameters of TDZ, FDZ, MA and M1, on the day before surgery, are presented in Table 3.1. The values for C_{max}, T_{max}, $t_{½}$ were within ranges reported by Smith *et al.* (*1983*). Data from these patients show that C_{max} and AUC values increased disproportionately and oral clearance values decreased significantly with dose. The same trend was observed for the C_{max} values of M1 but the difference was not statistically significant. Dose has no effect on T_{max} and $t_{½}$ values.

Compared to the day before, kinetic parameters, C_{max}, T_{max} and AUC_{0-4}, obtained prior to surgery for TDZ, FDZ, MA and M1 followed a similar pattern with respect to dose (Table 3.2) although the magnitude of these parameters tended to be lower.

Figure 3.2 shows the effect of CPB on TDZ, FDZ, MA and M1. Except TDZ, levels of FDZ, MA and M1 for the 60 mg and 90 mg treatment groups were not significantly different immediately before, during (15 min and 1 h), immediately after and up to 1 h after cessation of CPB. Although the kinetics of diltiazem were dose

dependent, the effects of CPB on the disposition of TDZ and FDZ, MA and M1 were similar between the two dosage regimens studied. Pooled data showed that TDZ levels were reduced significantly (~50 %) during the CPB procedure. The magnitude of FDZ level reduction was less (~20 %). The levels of MA and M1 were not changed with the onset of CPB. These meta s were still present in detectable quantities at sampling times 12 and 24 h.

Since CPB was initiated approximately 4 h after the morning dose, and since the whole procedure could last more than 7 h after the morning dose, a comparison of the AUC_{4-8} between the two treatment days was performed to evaluate the effects of CPB on diltiazem disposition (Table 3.2). Similar to the concentration profile changes, CPB triggered a marked reduction in TDZ AUC_{4-8} values (52 ± 13 and 54 ± 5% for the 60 and 90 mg treatment groups, respectively). The reduction of FDZ was minor and was only significant in the 90 mg group ($p = 0.029$). Similarly, AUC_{4-8} values of metabolites MA and M1 did not change significantly between the two treatment days.

The unbound fraction of diltiazem in plasma of the same patients, on both days, are presented in Figure 3.3. The binding of diltiazem was independent of concentration and the FF values recorded on the day before and prior to surgery were not significantly different for the two treatment groups. The unbound fraction of diltiazem was 0.43 ± 0.12 prior to CPB. After the onset of CPB, these values rose to a maximum of 0.83 ± 0.12 (significantly different from baseline) between 41 to 120 min (64.5 ± 26.2 min). Subsequently, diltiazem binding returned to baseline values 24 h after dosing (0.44 ± 0.13).

3.4 Discussion

This study presents a profile of the fate of oral diltiazem in patients undergoing cardiac bypass surgery. Our data show that 1) diltiazem displays dose dependent kinetics; 2) while total concentrations of diltiazem may be reduced at the onset of CPB, alterations in the protein binding offset this reduction resulting in unbound concentrations being maintained at levels similar to those seen the day prior to surgery; 3) M1 and MA levels are relatively insensitive to the CPB procedure.

Unbound fraction and $t_{½}$ values of diltiazem, on the day before surgery, were not altered by the dose. The similarity between the values suggests that distribution and systemic elimination of diltiazem are not dose related. This indicates that the reduction in oral clearance is due to non-linear first-pass effects. The mechanisms responsible for this phenomenon are not clear. It is very unlikely that the metabolic pathways responsible for MA and M1 disposition are involved because ratios of their AUC to TDZ or FDZ AUC remained the same. Saturation of other metabolic pathways and/or saturable tissue uptake may be responsible. A similar trend of decreased clearance was observed by Hoglund and Nilsson (*1989*) in a chronic study involving oral diltiazem administration in healthy subjects, although the dose dependent effect is more pronounced in this study. This variation may be partially due to age and disease state of the cardiac patients participated in the study.

Kinetic data during CPB are difficult to interpret because the condition of the patient is not stable. The abrupt decrease in TDZ level and AUC values, with the onset of CPB, is unlikely due to an increase in the elimination of diltiazem because the

corresponding FDZ data were not reduced to the same extent. In fact, after the initial reduction, FDZ levels increased with time during CPB and reached 96 ± 17% of the pre-CPB level within one hour. Furthermore, the metabolite levels and their AUC values do not change appreciably during the CPB procedure. These data suggest that the elimination of diltiazem and its metabolites is minimal during CPB and changes in their levels are results of hemodilution and redistribution.

Interestingly, when the six hour samples, collected at the end of a dosing interval, obtained from the day before surgery were compared with values obtained at the termination of CPB, which was 5.7 ± 0.9 h after dosing, the only difference observed were lower TDZ levels ($p = 0.002$ and 0.025 for the 60 mg and 90 mg q.i.d. treatment groups, respectively). These findings emphasizes the importance of measuring both the unbound and bound drug concentration when characterizing drug disposition during CPB.

The onset of the CPB procedure resulted in a rapid increase of FF for diltiazem and these values returned gradually to the baseline level at the end of the procedure. There are a number of factors which may influence drug binding during bypass surgery. The alteration in drug binding during CPB could be due to hemodilution (*Buylaert et al. 1989*), reduced temperature (*Kristensen and Gram 1982*), increased free fatty acids (FFA) level induced both by the administration of heparin and by the onset of CPB (*Wood et al. 1979, Jungbluth et al. 1989, Craig et al 1976, Hiriuchi et al. 1989, Holley et al. 1982*), plasticizer released from the bypass tubing, blood protein denaturation by the bypass equipment and possibly adsorption of proteins onto different parts of the CPB equipment (*Holley et al. 1982*). Our preliminary results showed that diltiazem binding

did not change at 28° C when compared to 37° C. FFA release, secondary to lipoprotein lipase activation, both *in vitro* and *in vivo*, has the potential to displace drugs from their binding sites (*Gartner and Vahouny 1966, Wood 1986*). Basic drugs such as disopyramide (*Hiriuchi et al. 1989*), propranolol (*Wood et al. 1979a, 1979b*) and lidocaine (*Brown et al. 1981*), which bind to AAG, are typical examples. Preliminary study in dogs showed that plasma diltiazem binding was not altered after intravenous heparin administration. The lack of FFA effect on diltiazem binding is interesting because this basic drug binds mainly to AAG (*Belpair and Bogaert 1990*). This discrepancy could be due to the binding of diltiazem to a different binding site on AAG. Protein adsorption onto the bypass equipment, particularly the oxygenator, may be important. In a kinetic study of alfentanil during CPB, Kumar *et al.* (*1988*) reported a 33% and 47% reduction in plasma albumin and AAG concentrations, respectively at the end of CPB. Hemodilution alone could not account for the reduction in the levels of these two plasma proteins because the priming solution contained albumin and hemodilution was approximately 25%. Their data suggest that plasma proteins, along with a portion of the bound drug were adsorbed onto the oxygenator and/or other part of the CPB equipment.

The plasma protein binding values for diltiazem in this study were lower than that reported in the literature (*Boyd et al. 1989*) (0.60 vs. 0.70 - 0.80). It is not clear whether concurrent medications and/or the presence of metabolites in plasma are responsible for the lower *in vivo* binding of diltiazem. We cannot attribute any age effect on the binding of diltiazem although our patient population is older, because the AAG

level measured (0.47-1.24 g/l) is within normal range of healthy subjects (0.51-1.20 g/l, *Belpaire and Bogaert 1990*).

The clinical implications of this study are that preoperative administration of diltiazem will provide a level of unbound drug equivalent to that normally present in a patient chronically taking the drug, even during CPB. This study emphasizes the importance of measuring both bound and unbound species of a drug when assessing the effect of CPB on pharmacokinetics.

3.5 Tables

Table 3.1 Pharmacokinetic parameters of diltiazem (total and unbound; TDZ, FDZ) and its *N*-demethyl and deacetyl metabolites (MA and M1) in patients receiving 60 and 90 mg diltiazem q.i.d. on the day before surgery (mean ± SD).

Kinetic parameters	60 mg q.i.d.				90 mg q.i.d.			
	TDZ	FDZ	MA	M1	TDZ	FDZ	MA	M1
C_{max} (ng/ml)	201 ± 43	85 ± 21	47 ± 18	32 ± 11	577 ± 95[a]	225 ± 46[a]	144 ± 34[a]	114 ± 75
T_{max} (h)	2.1 ± 0.8	2.4 ± 1.4	3.5 ± 1.7	3.1 ± 1.6	2.2 ± 1.0	1.3 ± 0.3	1.3 ± 0.5	3.4 ± 1.9
$t_{½}$ (h)	4.7 ± 2.1	5.3 ± 2.9	4.8 ± 2.6	5.6 ± 2.3	4.0 ± 1.1	3.8 ± 1.2	5.2 ± 2.2	10.4 ± 8.3
AUC_{0-4} (ng.h/ml)	662 ± 178	265 ± 72	153 ± 65	97 ± 30	1818 ± 323[a]	677 ± 81[a]	491 ± 108[a]	393 ± 264[a]
AUC_{4-8} (ng.h/ml)	470 ± 83	199 ± 56	132 ± 44	83 ± 29	1285 ± 294[a]	487 ± 118[a]	446 ± 118[a]	367 ± 236[a]
AUC_{0-6} (ng.h/ml)	945 ± 246	377 ± 95	223 ± 85	139 ± 41	2579 ± 460[a]	968 ± 132[a]	737 ± 179[a]	589 ± 380[a]
Cl/F (l/h)	66 ± 14	170 ± 54			36 ± 6.9[a]	94 ± 13[a]		

[a] *significantly different from the 60 mg dosing schedule ($p < 0.05$).*

Table 3.2 Pharmacokinetic parameters of diltiazem (total and unbound; TDZ, FDZ) and its *N*-demethyl and deacetyl metabolites (MA and M1) in patients receiving 60 and 90 mg q.i.d. on the day of surgery (mean ± SD).

Kinetic parameters	60 mg q.i.d.				90 mg q.i.d.			
	TDZ	FDZ	MA	M1	TDZ	FDZ	MA	M1
C_{max} (ng/ml)	182 ± 69	79 ± 26	49 ± 7	33 ± 14	439 ± 70[ab]	221 ± 77[a]	164 ± 77[a]	99 ± 62
T_{max} (h)	1.7 ± 0.7	1.6 ± 0.2	1.8 ± 0.8	1.2 ± 0.5[b]	1.6 ± 0.6	1.6 ± 0.6	2.1 ± 1.1	1.3 ± 0.5
AUC_{0-4} (ng.h/ml)	536 ± 159[b]	249 ± 86	146 ± 44	97 ± 32	1282 ± 251[ab]	607 ± 112[a]	402 ± 107[a]	318 ± 200
AUC_{4-8} (ng.h/ml)	224 ± 64[b]	153 ± 37	170 ± 143	76 ± 34	588 ± 122[ab]	388 ± 81[ab]	353 ± 232	250 ± 130[a]

[a] *significantly different from the 60 mg dosing schedule ($p < 0.05$).*

[b] *significantly different from the previous day values ($p < 0.05$).*

3.6 Figures

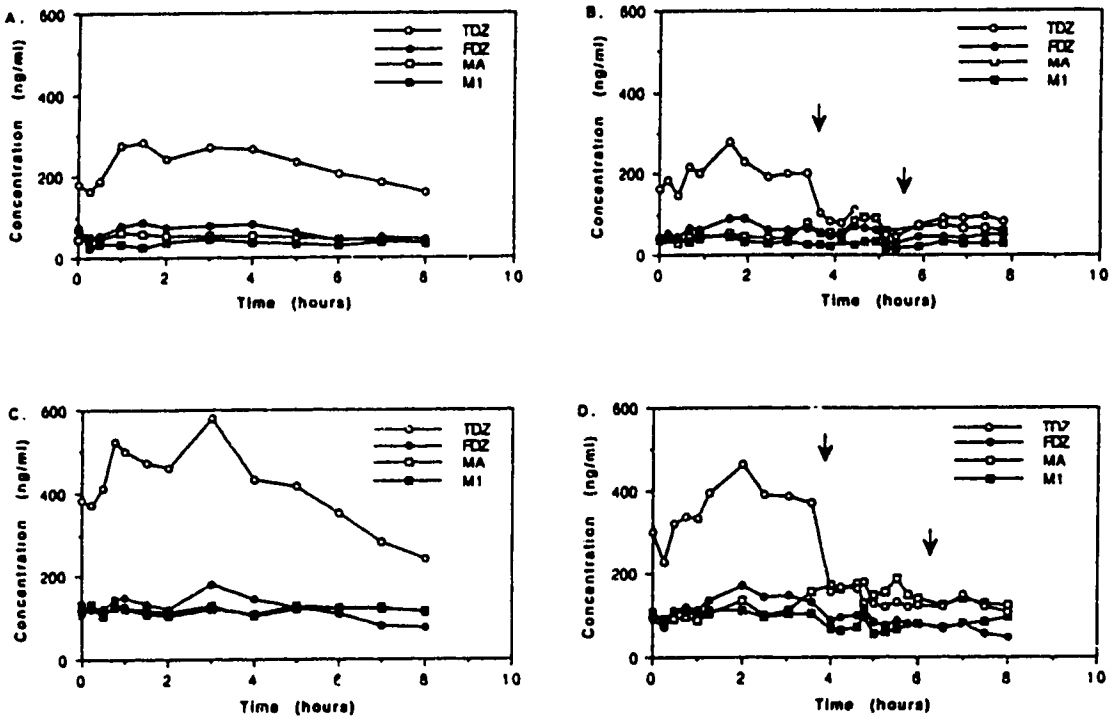

Figure 3.1 Serum concentration versus time profiles of diltiazem (total and unbound; TDZ, FDZ) and its *N*-demethyl and deacetyl metabolites (MA and M1) in two patients on both days. Day before surgery (A), day of surgery (B) in a patient receiving 60 mg diltiazem q.i.d. and day before surgery (C), day of surgery (D) in a patient receiving 90 mg diltiazem q.i.d. Arrows indicate the beginning and end of bypass procedure.

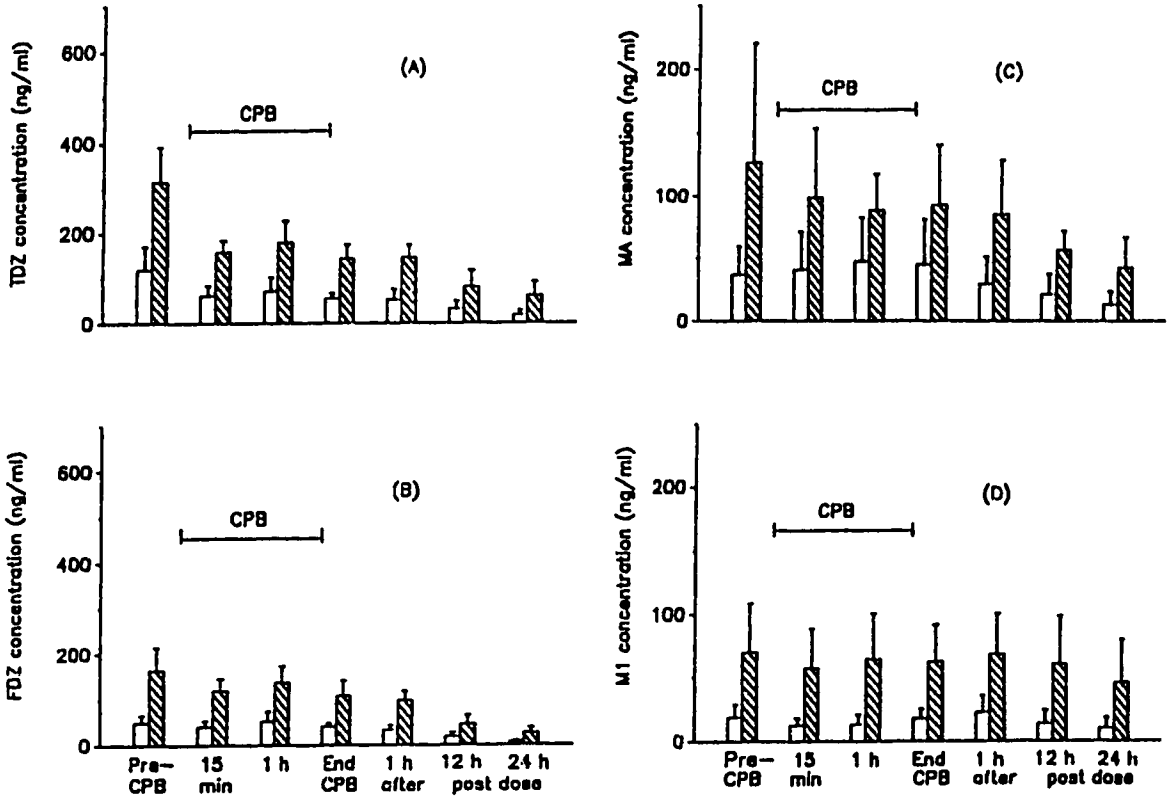

Figure 3.2 Effect of CPB on serum concentration of diltiazem, total (TDZ) (A) and unbound (FDZ) (B), MA (*N*-demethyl metabolite) (C) and M1 (deacetyl metabolite) (D). Open bars represent diltiazem dosed at 60 mg q.i.d. and hatched bars at 90 mg q.i.d. Error bars represent SD.

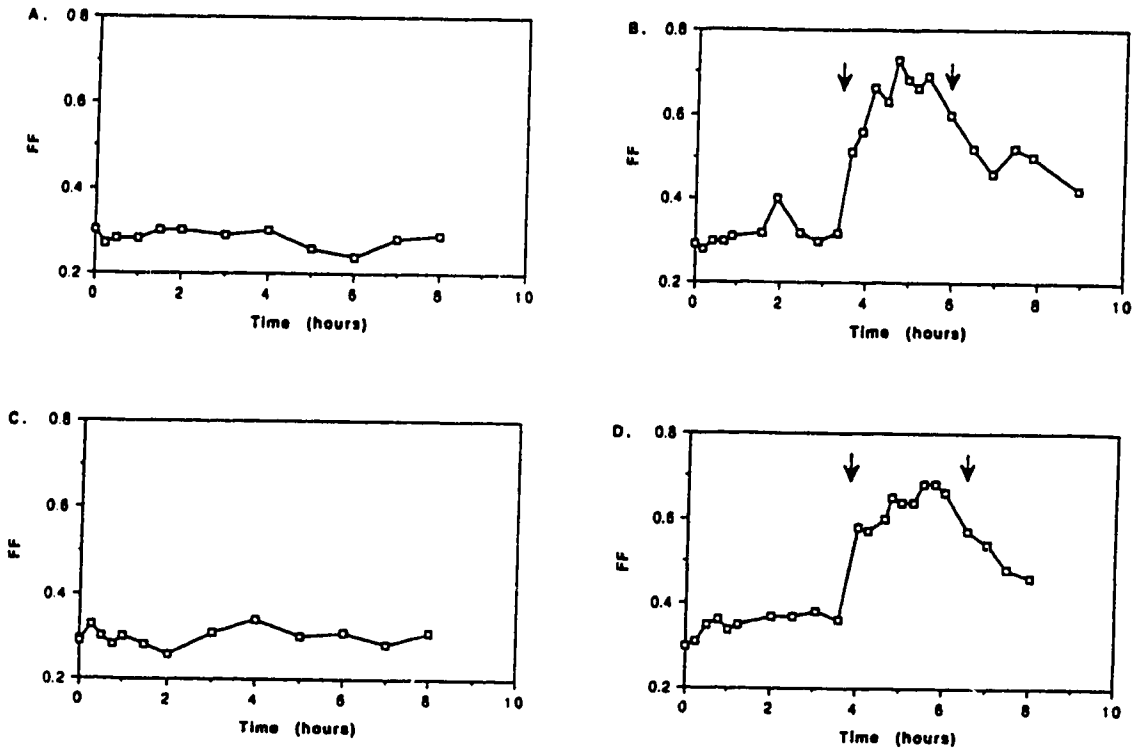

Figure 3.3 Unbound fraction (FF) profiles of diltiazem on both days measured in the same two patients. Day before surgery (A), day of surgery (B) in the patient receiving 60 mg diltiazem q.i.d. and day before surgery (C), day of surgery (D) in the patient receiving 90 mg diltiazem q.i.d. Arrows indicate the beginning and end of bypass procedure.

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4. MECHANISMS OF TIME-DEPENDENT KINETICS OF DILTIAZEM IN THE ISOLATED PERFUSED RAT LIVER[3]

4.1 Introduction

Drugs with tertiary amino group such as diphenhydramine and its analogs can inactivate *N*-dealkylating isozymes of P450 by forming stable metabolic intermediates (*Bast and Noordhoek 1982, Bast et al. 1984, 1990*). This phenomenon was postulated to be the main reason for the time dependent reduction of clearance (time-dependent kinetics) of these drugs, during repetitive administration (*Labout et al. 1982*). Time-dependent kinetics were also observed for cardiovascular drugs with a tertiary amino group, such as diltiazem, lidocaine and verapamil (*Bauer et al. 1982, Hoglund and Nilsson 1989, Hung et al. 1988, Lennard et al. 1983, Meredith et al. 1985, Ochs et al. 1980, Schwartz et al. 1985, Smith et al. 1983, Tam et al. 1987*), possibly due to enzyme inactivation.

When diltiazem is given as a single oral dose in healthy subjects, the AUC increases proportionally with dose (*Kinney et al. 1981*). The AUC and steady state diltiazem concentration after multiple dosing increases more than the predicted values from a single dose study (*Hoglund and Nilsson 1989a, Hung et al. 1988, Smith et al. 1983*). From this it can be deduced that the time-dependent effect is not due to saturation of metabolism. It has been shown that lidocaine is metabolized mainly by *N*-dealkylation

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[3] A version of this chapter has been submitted for publication in *Drug Metab Dispos* (1992).

and can inactivate certain P450 isozymes, giving rise to time-dependent kinetics (*Saville et al. 1989*). Diltiazem undergoes extensive first pass metabolism in the liver (*Buckley et al. 1990, Meshi et al. 1971*) and the major phase I metabolic pathways are N-dealkylation and deacetylation in the human (*Buckley et al. 1990, Hoglund and Nilsson 1989b, Yeung et al. 1990*). It is possible that a similar mechanism of enzyme inactivation is responsible for the observed time-dependent kinetics of diltiazem.

Enzyme inactivation has significant clinical implications when the drug exerting a time-dependent effect has a narrow therapeutic index or when it is coadministered with drugs sharing common enzymatic pathway(s) for elimination. The effect of the enzyme inactivation may persist even after the drug is cleared from the body and may affect the kinetics of other drugs (*Saville et al. 1989*).

Additional factors such as change in hepatic blood flow, change in plasma protein and tissue binding, product inhibition may contribute to the time-dependent kinetics of diltiazem. In healthy subjects after multiple oral doses, diltiazem does not alter hepatic blood flow as evident from unchanged indocyanine green clearance (*Bauer et al. 1986*). In these subjects diltiazem reduced the antipyrine clearance by 6-24%, suggesting time-dependent reduction in clearance was not due to a change in hepatic blood flow. Changes in binding of serum protein is an unlikely contributing factor to the time-dependent phenomenon because the binding ($\sim$70%) remains constant in the therapeutic concentration range of 0.1 to 1 μg/ml (*Belpaire and Bogaert 1990*). Furthermore, the serum binding of diltiazem in patients, who were on chronic diltiazem therapy (*Finegan*

et al. 1992, Hung et al. 1988), were similar to the single dose data (*Boyd et al. 1989, Hung et al. 1988*).

The objective of this study was to investigate the mechanisms contributing to the time-dependent kinetics of diltiazem. We selected the rat as an animal model because it has been used extensively to study the metabolism and pharmacokinetics of diltiazem (*Meshi et al. 1971, Piepho et al. 1982, Yeung et al. 1990*). The metabolism of diltiazem in rats is comparable to that of humans (*Meshi et al. 1971*). In addition, this animal model has been used to study enzyme inactivation and competitive inhibition between drugs, some of which are tertiary amines (*Bruck et al. 1990, Lennard et al. 1983, Maenpaa et al. 1989, Masubuchi et al. 1992, Saville et al. 1989, Schneck and Pitchard 1981, Tam et al. 1987*). The use of a single-pass IPRL system permits the study of these two factors independently. Steady state drug and metabolite levels reflect dose-dependant metabolism and product inhibition, while time to steady state is determined by other mechanisms such as tissue binding and/ or enzyme inactivation (*Tam et al. 1987*).

4.2 Materials and Methods

4.2.1 Chemicals and Reagents

Diltiazem and the metabolites: M1, M2, M4, M6, and M4-NO as hydrochloride salts, and MA as its fumarate salt were kindly supplied by Nordic Merrell Dow Research (Laval, Quebec, Canada). Radiolabeled diltiazem (methyl-^{3}H diltiazem) was obtained

from Amersham Canada Ltd (Oakville, Ontario, Canada). All other reagents were of analytical grade.

4.2.2 Liver Perfusion

Male Sprague-Dawley rats (Biosciences Animal Services, University of Alberta, AB, Canada), weighing 210-250 g, maintained on rat chow (Wayne Rodent Plox 8604-00, Continental Grain company, Chicago, IL, USA) were used in this study. The animals were housed in the Dentistry-Pharmacy animal facility for at least three days before an experiment. An alternating 12 h light (6 a.m.-6 p.m.) and dark (6 p.m.-6 a.m.) cycle was maintained. Food and water were supplied *ad libitum*. The methods of liver isolation and perfusion were as previously reported (*Miller 1973, Tam et al. 1987*) with a few modifications. Methoxyflurane (Pitman-Moore, Mississauga, Ontario, Canada) was used to anesthetize rats before surgery and the portal vein was cannulated with an intravascular over-the-needle teflon catheter (Quick-Cath, Baxter Healthcare Corporation, Dearfield, IL, USA). The viability of the liver was monitored by O_2 consumption (YSI model 5300 biological oxygen monitor micro system, Yellow Springs, OH, USA), constant perfusion pressure (Rosemount Model 1151 differential pressure transducer, Rosemount Inc., Minneapolis, MN, USA), standard liver function tests (aspartate and alanine aminotransferase levels in the perfusate effluent) before and after the experiment and by its physical appearance. Furthermore, the stable concentrations of diltiazem and its metabolites in the effluent perfusate at steady sate during diltiazem infusion served as an indicator of liver viability.

The liver was allowed to equilibrate for 20 min before diltiazem, dissolved in the perfusing media, was perfused. Diltiazem (2-100 μM) was infused through the portal vein up to 120 min to achieve steady state concentration of diltiazem and its metabolites. The higher infusion concentration of diltiazem was selected to mimic a high portal vein concentration after oral dosing (*Semple et al. 1990*). The infusion time is sufficient for enzyme inactivation (*Tam et al. 1987*). Effluent from the thoracic inferior vena cava was collected at 0, 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90, 105, 120 min for measurement of diltiazem and its metabolites concentration (outlet concentration, C_{out}). Inlet samples were taken at 0, 2, 8, 12, 24, 51, 81 and 98 min to determine the inlet concentration of diltiazem (C_{in}). Samples ($\sim$5 ml) were collected on an ice bath. Hydrochloric acid (3N, 25 μl) was added to each sample to give a pH $\sim$5.0. These samples were stored at -25°C and HPLC analysis was performed within two weeks. Diltiazem and its metabolites were stable under these conditions. At the end of each experiment, the liver was blotted dry and its weight was determined.

4.2.3 Stop-Infusion Studies

This experiment was designed to evaluate binding and enzyme inactivation of diltiazem in the isolated perfused rat liver. Livers from five different rats were infused with C_{in} of 38.0 $\pm$ 4.4 μM up to 40 min. Then the drug and the metabolites were allowed to wash out by perfusing the liver with plain buffer for a period of 30 min. Effluent samples were collected at 1, 5, 10, 15, 20 and 30 min of the washout period. After the washout period, the same rate was infused again for another 40 min. During

both infusions, effluent samples were collected at 1, 3, 5 min and every 5 min thereafter up to 40 min.

4.2.4 Radiolabeled Diltiazem Infusion Studies

To investigate the reversible and irreversible binding of diltiazem to liver proteins and tissues, livers from six rats were infused with a mixture of labeled and unlabeled diltiazem at two different C_{in} values (35.5 $\pm$ 3.2 and 67.2 $\pm$ 3.4 μM, n=3 in each group) until steady state was achieved. The bile duct was cannulated with a PE 10 catheter before the catheterization of the portal vein. Perfusate from the liver was collected, as described above, for radioactivity measurement. Bile and the posterior vena cava effluent were also collected. The liver was washed with the perfusing buffer for 78 min and the washouts were collected periodically for analysis. The washed livers were homogenized and microsomes were prepared by procedures described by von Bahr *et al.* (*1980*), except that the supernatant of the liver homogenate were centrifuged again at 10,000 *g*. The extraction procedure described by Nakagawa *et al.* (*1979*) was employed to measure the irreversible binding of labeled diltiazem or a reactive metabolite to liver tissues, microsome and cytosol (supernatant after 100,000 *g* centrifugation) except that each extraction with methanol-ether mixture was performed twice. After 10 extractions, no further radioactivity could be removed from the pellet. Subsequently the pellet was dissolved in 0.5 ml of 1 N NaOH, mixed with 16 ml of ScintiVerse™ (Fisher Scientific, Nepean, Ontario, Canada) and the radioactivity was counted in a Wallac 1410

liquid scintillation counter (Fisher Scientific, Ontario, Canada). Protein concentration was determined using the Sigma protein assay kit (St. Louis, MO, USA).

4.2.5 Measurement of Diltiazem and Metabolites

The concentrations of diltiazem and its metabolites in the liver perfusate were analyzed using HPLC. The HPLC system (Waters Instruments, Mississauga, Ontario, Canada) consisted of a Model 501 pump, a Model 710B automatic injector and a Model 441 UV detector set at 214 nm. Samples were injected directly onto a C_{18} reverse-phase Novapak cartridge (Waters Instruments, 4 μm particle size, 10 cm x 8 mm I.D.). The mobile phase consisted of an aqueous solution of 0.092% H_3PO_4 and 0.2% triethylamine (74 parts, v/v) and acetonitrile (26 parts, v/v) and the flow rate was 2 ml/min. Bupivacaine HCl (Sigma Chemicals Co.) was used as internal standard. Calibration curves were linear over the concentration range studied (10-2000 ng/ml). The intra and inter-day coefficients of variation for diltiazem and its metabolites were less than 10%.

4.2.6 Data Analysis

Steady state concentration (C_{ss}, μM) of diltiazem and the metabolites and the time to reach the steady state condition (T_{ss}, min) were determined according to the procedure reported by Saville *et al.* (*1989*). This statistical test was also applied to evaluate the presence of a peak in metabolite profiles. Steady state extraction ratio (E), hepatic drug clearance Cl_H (ml/min/g liver) and material balance (MB) were calculated using the following equations:

$$E = \frac{C_{in} - C_{out}}{C_{in}} \qquad (1)$$

$$Cl_H = Q \times E \qquad (2)$$

$$MB = \frac{C_{known} \times 100}{C_{in}} \qquad (3)$$

where, Q is the perfusion flow rate (ml/min) and C_{known} (μM) is the total concentration of diltiazem and its known metabolites in the effluent. The total accumulation of diltiazem and its metabolites in the liver was determined from the area above the labeled flux curve (QxC_{out}) *vs* time up to the steady state. Also the area under the flux curve *vs* time (AUFC) during washout in radioactive diltiazem infusion and stop-infusion experiments was determined. These areas and the half-life for washout of diltiazem and its metabolites from the liver were calculated using the computer program LAGRAN (*Rocci and Jusko 1983*). Partition coefficients (K_{TW}) of diltiazem and the metabolites in the liver tissue *vs* buffer solution in the sinusoid were determined by using the following equation:

$$K_{TW} = \frac{AUFC\,\rho}{C_{ss}\,W f_t} \qquad (4)$$

where, W is the weight of the liver (g), ρ is the density of the liver (g/ml) and f_t is the fraction of the liver volume occupied by liver tissues. Because the vascular and interstitial region of the liver is ~15 percent of the total liver volume (*Brauer 1963,*

Goresky 1963), f_t was assumed to be 0.85. The density of the liver ρ was assumed to be 1 g/ml (*Gray et al. 1987*).

4.2.7 Statistics

Data are reported as mean ± SD. The paired t-test was used to test any differences in C_{in}, E, Cl_H, MB and T_{in} between the two infusions in the stop-infusion experiment. The unpaired t-test was used to test any difference in irreversible binding and total binding data between the two C_{in}s. The level of significance was set at p=0.05.

4.3 Results

The concentration time courses of diltiazem and its metabolites after continuous infusion are shown in Figure 4.1. Diltiazem and its metabolites are mainly metabolised by deacetylation, *O*-demethylation, *N*-demethylation and *N*-oxidation. There is a gradual reduction of extraction of diltiazem with time; both the drug and its metabolites reach a steady state at around 60 min. *N*-Demethyldiltiazem, although a minor metabolite (~3% of C_{in} at steady state), interestingly increases to a maximum concentration at around 7-20 min and declines to a steady state in most of the livers studied (23 out of 34). This behaviour unexpectedly does not follow the time course of diltiazem. At steady state, relative contributions of M1, M2, M4, M6 and M4-NO to mass balance were 19.5 ± 1.8, 6.9 ± 2.9, 13.7 ± 2.4, 8.1 ± 2.1 and 3.2 ± 1.3%, respectively of the C_{in} of 10.4 ± 0.9 μM. This sequence reveals that metabolites were formed by different metabolic

pathways in the decreasing order: deacetylation >> *O*-demethylation > *N*-demethylation >> *N*-oxidation. Unchanged diltiazem accounted for 12.5 ± 8.9% of the infused diltiazem in the steady state effluent at this C_{in}.

The formation rate of primary metabolites M1 and MA by deacetylation and N-demethylation, respectively were linear up to a diltiazem C_{out} of 26 μM (Figure 4.2). Similar plots for secondary metabolites (M2 and M4) and tertiary metabolites (M4-NO and M6) were not constructed because the relative contribution of the precursors in their formation was not known.

The T_{ss} decreased as the C_{out} increased and the plot of T_{ss} vs C_{out} tends to come to a steady value of ~ 15 min at C_{out} values greater than 15 μM (Figure 4.3). The material balance (MB) with time are shown in Figure 4.4 for two different C_{in} values. At low C_{in}, around 40% of the drug is unaccounted for at steady state, suggesting the presence of other unidentified pathways of metabolism. These pathways are probably saturable because material balance (within ± 5%) can be achieved at high C_{in}. Steady state E declined from 0.98 to 0.73 when C_{out} increased from 0.03 to 26.34 μM (Figure 4.5). Calculation of the metabolites as a percentage of C_{in} suggest that the reduction in E may be related to the saturation of *O*-demethylation and other unknown metabolic pathways. Total deacetylated and *N*-oxidized metabolites remained constant in the concentration range studied, while total *N*-demethylated metabolites decreased at C_{in} > 55 μM.

In the stop-infusion experiment, profiles of diltiazem and the metabolite concentration versus time were similar during both infusions, separated by a washout period of 30 minutes (Figure 4.6), with the exception of the concentration versus time

profile of metabolite MA. On both occasions, diltiazem rose slowly to steady state. In all livers studied, MA showed a characteristic rise to a higher than steady state concentration prior to a fall to the steady state condition during the first infusion (Figure 4.6B); this peak was statistically verified in three livers. C_{ss}, E, Cl_H, MB and T_{ss} for diltiazem during the second infusion were not significantly different when compared with those of the first infusion (Table 4.1). Diltiazem and MA were detected up to 15 min in the washouts, while the other metabolites were still detected after 30 min of washout. The half-life during washout was shortest for diltiazem and longest for M6 (Table 4.2) and linear regression indicates that this parameter is related to partition coefficient ($r = 0.88$).

During infusion of the mixture of labeled and unlabeled diltiazem, steady state was reached in 15 to 20 minutes (C_{in}s; 35.5 $\pm$ 3.2 and 67.2 $\pm$ 3.4 μM). At steady state, more than 90% of the radioactivity was recovered in the effluent from the liver. Excretion of radioactivity through bile accounted for only 2 to 3% of the infused radioactivity. Excretion through the inferior vena cava near the right kidney was also negligible (1.6 - 2.6%). Total recovery of the infused ^{3}H-diltiazem radioactivity was obtained in this experiment. The half-life for radioactivity washout from the liver was 16.1 $\pm$ 2.8 min and radioactivity was observed in the outlet even at 78 min after drug infusion ceased, suggesting that diltiazem and/or its metabolites were tightly bound to liver tissue.

Diltiazem and/or its metabolites were irreversibly bound to different liver proteins and this binding was not different at the two C_{in} studied. The mean total amount of

unlabeled diltiazem and its metabolites (calculated as diltiazem) that were irreversibly bound to whole liver, hepatic microsomal and hepatic cytosolic proteins were 24.5 ± 6.6, 48.8 ± 11.8 and 23.7 ± 5.8 pmol/mg of protein respectively.

The total amounts of unlabeled diltiazem and its metabolites bound (reversible and irreversible) and/or distributed in liver tissues, as calculated from the area above the labeled flux curve *vs* time up to steady state, were 669.5 ± 156.5 and 974.2 ± 99.2 nmol/g of liver at C_{in} of 35.5 ± 3.2 and 67.2 ± 3.4 μM respectively and are significantly different ($p=0.047$). Similar values were obtained from the AUFC during washout. These data show that irreversible binding is negligible compared to both reversible binding and distribution of diltiazem and its metabolites into liver tissues. The overall partition coefficient for diltiazem and its metabolites, K_{TW} calculated from radioactivity data using equation (4) were 23.6 ± 4.8 and 19.3 ± 1.0 at the two C_{in}s studied; these values were not significantly different.

Viability parameters monitored in a representative IPRL study are shown in Figure 4.7. These parameters were found to be stable throughout each study; maximum variation was less than 15%. The rate of oxygen consumption was within the normal range reported (*Bloxam 1973, Gores et al. 1986, Tam et al. 1987, Yau 1986*). These conditions could be maintained for at least 150 min after catheterization, suggesting the liver was viable over this period of time.

4.4 Discussion

In this study we found that diltiazem exhibited time-dependent kinetics in the IPRL model due to a slow approach to its steady state concentration. The mechanisms of the time dependent kinetics may be attributed mainly to reversible binding of diltiazem to liver tissues. Inactivation of enzyme(s) involved in *N*-dealkylation plays a minor role. These conclusions are derived from the results of 1) a strong reversible binding of diltiazem and/or its metabolites to liver tissue as shown in both the stop-infusion and radioactive diltiazem infusion studies. 2) a maximum in the concentration profile of *N*-demethylated diltiazem MA that was not observed upon reinfusion of drug after washout. 3) irreversible binding of diltiazem and/or metabolites to whole liver, hepatic microsomal and hepatic cytosolic proteins which is much lower than the reversible binding.

Deacetylation and *N*-demethylation were the primary metabolic pathways detected for diltiazem in this study, the former being the major pathway. Both metabolic pathways were linear (Figure 4.2), suggesting that saturation of metabolic enzymes did not play an important role in the time dependent kinetics of diltiazem and other factors must be considered.

The time required to achieve steady state concentration of diltiazem is long. Assuming that the liver is a well mixed compartment, the mean residence time of perfusate is between 20-60 seconds depending on the medium and flow rate (*Gray et al. 1987*). This time is much shorter than the time to steady state for diltiazem in our studies. The longer T_{ss} for diltiazem may be partially due to its tissue binding and distribution. Capacity-limited tissue binding has been suggested to lidocaine for which

a similar trend of prolonged time to steady state was observed (*Tam et al. 1987*). The mass balance study shows that diltiazem is not fully accounted for in the early part of infusion (Figure 4.4), especially when the unidentified metabolites become insignificant at higher C_{in}. Also there is a characteristic sigmoidal rise of diltiazem concentration with time (figs. 1 and 6). These observations are consistent with the binding of diltiazem to the liver tissue. Gray *et al.* (*1987*) observed a similar trend when fitting LID data to different models. The drug and its metabolites may have a high affinity for the cellular region of the liver which can act as a large reservoir for drugs. During initial drug administration, it can take a long time to fill the reservoir (*Saville et al. 1992*). The high affinity of diltiazem and its metabolites for liver tissue is reflected by their high partition coefficients (Table 2) and may explain the longer time to achieve steady state. The time to achieve steady state decreases at higher C_{in} of diltiazem (Figure 4.3). These data and the total amount of diltiazem and its metabolites bound to the liver, as measured in the labeled diltiazem studies suggest that the hepatic tissue binding is saturable. The short rise in MA concentration to a maximum and its subsequent decline to steady state was unexpected because diltiazem concentration was still rising during this period. If tissue binding were the only cause of time-dependency, the MA level would follow that of diltiazem. This observation is not unique for diltiazem. The phenomenon of maximum was also observed in the concentration profile of MEGX, the *N*-monodeethylated metabolite of lidocaine (*Tam et al. 1987*) and was attributed to enzyme inactivation (*Saville et al. 1989*). In the case of orphenadrine, a tertiary amine, enzyme inactivation occurred during *N*-dealkylation (*Bast and Noordhoek 1982*). It appears that many

alkylamine-substituted drugs inhibit their own metabolism by inactivating P450 isozymes (*Bast and Noordhoek 1982, Bast et al. 1984, Bast et al. 1990, Gray and Tam 1991, Larrey et al. 1983, Murray and Field 1992*).

The stop-flow infusion experiment was performed to test the nature of binding and any persistent alteration in the metabolism of diltiazem. If enzyme inactivation was mainly responsible for the time-dependent kinetics, then diltiazem would come to a steady state rapidly with a second exposure. In contrast, if the time dependent behaviour was mainly due to reversible binding, there would be no difference in the time to steady state in the second exposure. Diltiazem showed both reversible binding and inactivation of N-dealkylating enzyme in the stop-infusion experiment. The former is deduced from the similar pattern for the rise of diltiazem to steady state during the two intermittent infusions (Figure 4.6). The latter is shown by the absence of the maximum in MA concentration-time profile during the second infusion. The initial absence of diltiazem in the effluent (1-3 min) immediately after the start of the second infusion can be attributed to reversible binding (Figure 4.6). Kinetic parameters for the two infusion stages were statistically equivalent suggesting no significant effect of diltiazem pretreatment on the rat liver.

Detectable levels of diltiazem and metabolites were found in the effluent during washout for at least 10 min after cessation of drug infusion. The time scale of mixing of a compound within the liver is only 20 to 30 seconds (using total liver volume and no tissue transport limitation) and the organ should be free of drug within 2 to 3 min (*Saville 1989*). Our observations suggest that diltiazem and its metabolites are subject to

retention within the liver. Partition coefficient data (Table 4.2) also indicate that liver tissue acts as a reservoir for these lipophilic compounds. A calculation based on partition coefficient of diltiazem shows that at the steady state condition there is ~45 times more diltiazem in the liver tissue than in the sinusoid, assuming tissue volume is five times higher than the extracellular volume. The high affinity of diltiazem and its metabolites for the liver tissue explains long washout half-lives (Table 4.2) of diltiazem and its metabolites from the rat liver.

The reversible binding of diltiazem is stronger than LID binding based on a comparison of the washout times of ~10-15 min for diltiazem versus ~5 min for LID (*Saville et al. 1989*). Also the ratio of the rate constant for cellular uptake to the rate constant for cellular release (equivalent to K_{TW}, as the reaction rates are small relative to rates of transport) of lidocaine and its metabolites MEGX and 3-hydroxy-lidocaine (3-OHLID) was 5.0 ± 1.3 (*Saville et al. 1992*). This value is much lower than the K_{TW} of diltiazem and its metabolites (table 2), suggesting that diltiazem and its metabolites have higher affinity for the liver tissue. It is interesting to note that both diltiazem and lidocaine have identical pK_a values (7.7) and octanol/water partition coefficients ($\log_{10}$ P = 2.3) (*Bokesch et al. 1986, Hermann and Morselli 1985*). These data suggest that partitioning of diltiazem and its metabolites into the liver tissue is due to their affinity for the binding sites in liver tissue. If solubility in cell membranes was the only mode of tissue retention, then diltiazem and lidocaine would have equivalent partition ratios.

The infusion studies with radiolabeled diltiazem have further clarified the nature of diltiazem's time-dependent kinetics. From the calculated $t_{1/2}$ for radioactivity during

washout, it is apparent that diltiazem and/or its metabolites are tightly bound to liver tissues. Also when the hepatic microsomal pellets were washed, 16%, 13% and 11% of the total radioactivity was removed in the first, second and third washing, respectively, suggesting strong binding to microsomal protein.

Diltiazem and/or its metabolites are irreversibly bound to both microsomal and cytosolic proteins, specially to the former. The irreversible binding of diltiazem to cytosolic protein is interesting since cytosolic enzymes, like microsomal enzymes, are involved in the biotransformation of both endogenous and exogenous compounds (*Cesarone et al. 1987, Corbett et al. 1988, Gillette 1971, Kundu et al. 1991, Lake and Gangolli 1981*).

Changes in the blood flow rate and plasma protein binding can be ruled out as a cause of time-dependent kinetics in isolated perfused rat liver since the perfusion rate was kept constant and there was no protein in the perfusion medium. The liver was viable up to the end of the experiments as monitored by the steady oxygen consumption, hydrostatic pressure, enzyme levels and general appearance of the liver. The maintenance of steady state level of drug and metabolites is a further indication of the viability of the liver throughout the experiment.

From this study, we conclude that the time dependent kinetics of diltiazem in rat is mainly due to reversible tissue binding. In rat liver, the *N*-demethylation of diltiazem is a minor pathway and the probable contribution of enzyme inactivation to time-dependent kinetics may be small compared to the contribution of binding. In human, however, the major metabolic pathway for diltiazem metabolism is *N*-demethylation and

consequently enzyme inactivation, as observed in rat, may play a significant role in time-dependant kinetics of diltiazem.

4.5 Tables

Table 4.1 Comparison of kinetic parameters of diltiazem at two infusions during stop-infusion experiments (Cin = 38.00 ± 4.44 μM, n = 5).

Pharmacokinetic parameters	First infusion	Second infusion
C_{ss} (μM)	4.59 ± 1.81	5.01 ± 1.34
E	0.88 ± 0.04	0.87 ± 0.02
Cl_H (ml/min/g liver)	2.68 ± 0.09	2.63 ± 0.04
MB (%)	75.83 ± 9.21	76.06 ± 8.44
T_{ss} (min)	25.00 ± 3.54	21.00 ± 2.24

Table 4.2 Half-live and partition coefficient of diltiazem and its metabolites calculated during washout period in stop-infusion experiments (n = 5).

	Diltiazem	MA	M1	M4	M2	M4-NO	M6
$t_{1/2}$ (min)	1.82 ± 0.36	2.42 ± 0.47	5.36 ± 0.96	6.59 ± 1.20	11.10 ± 4.11	11.34 ± 6.09	14.86 ± 3.72
K_{TW}	8.87 ± 0.77	14.69 ± 1.34	18.52 ± 1.66	25.58 ± 3.50	34.51 ± 6.19	34.34 ± 5.88	76.02 ± 22.43

4.6 Figures

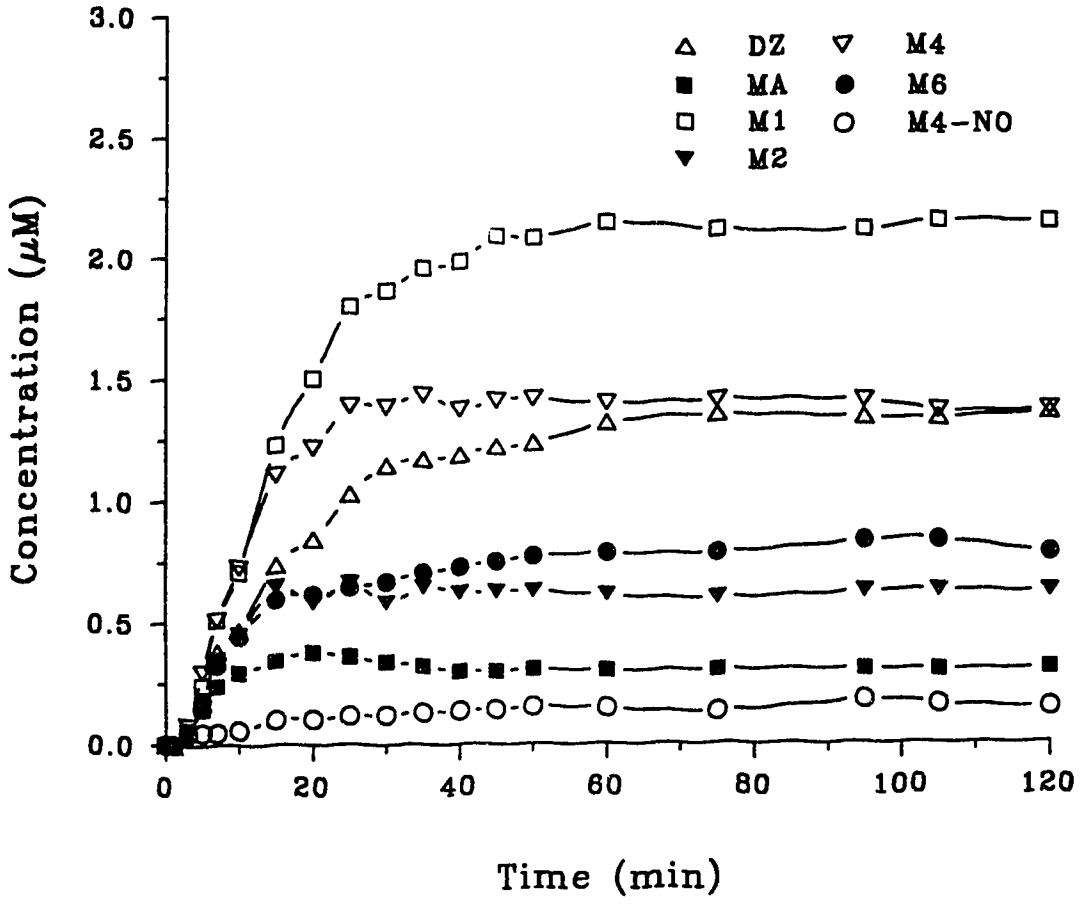

Figure 4.1 Concentration *vs* time profiles of diltiazem (DZ) and its metabolites in the effluent from an isolated rat liver (inlet DZ concentration, 10.3 μM).

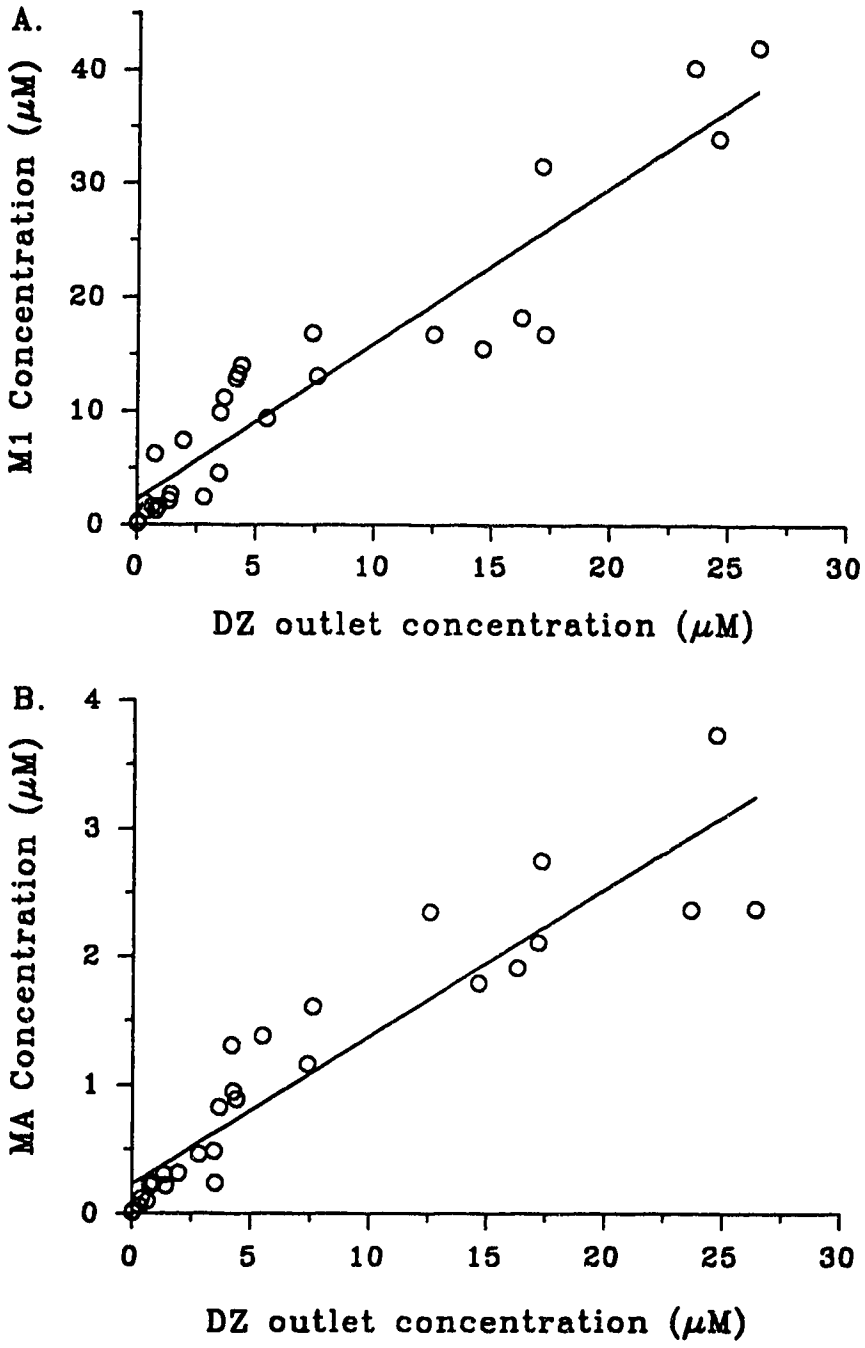

Figure 4.2 Steady state M1 (A) and MA (B) concentrations *vs* outlet diltiazem (DZ) concentration.

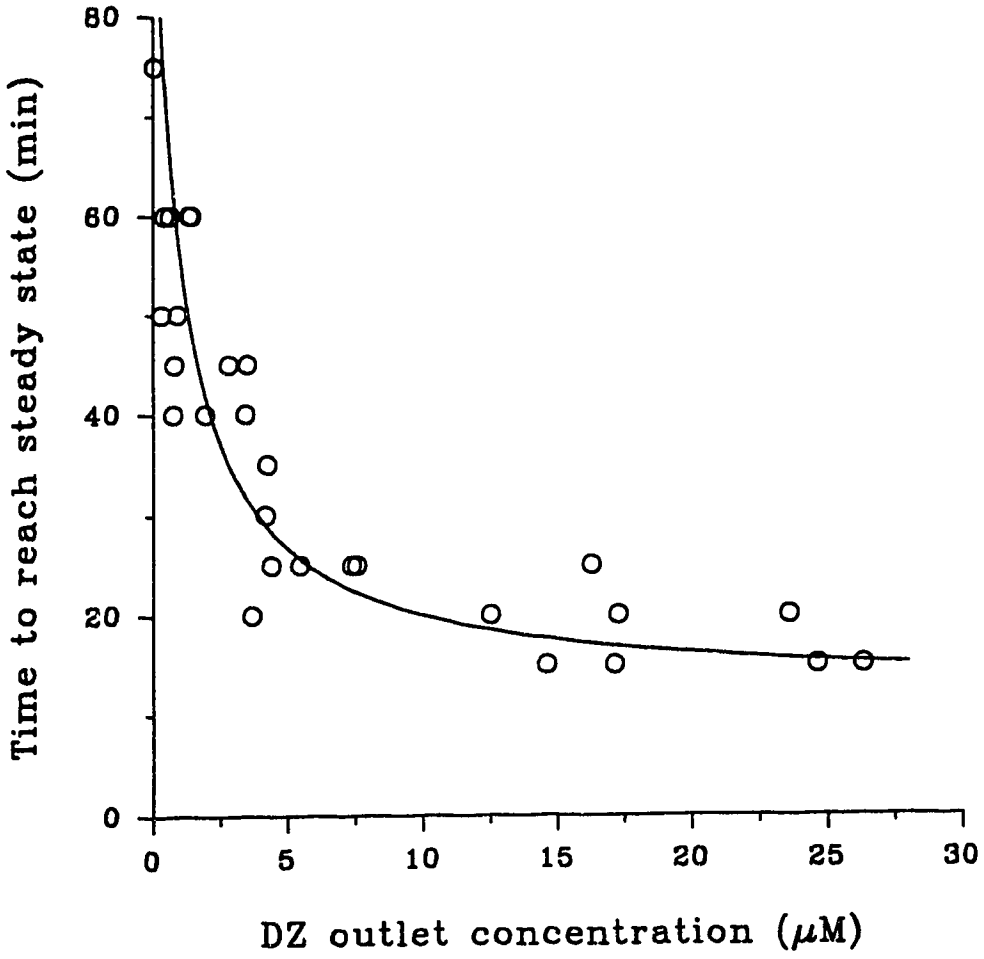

Figure 4.3. Time to reach steady state *vs* outlet diltiazem (DZ) concentration.

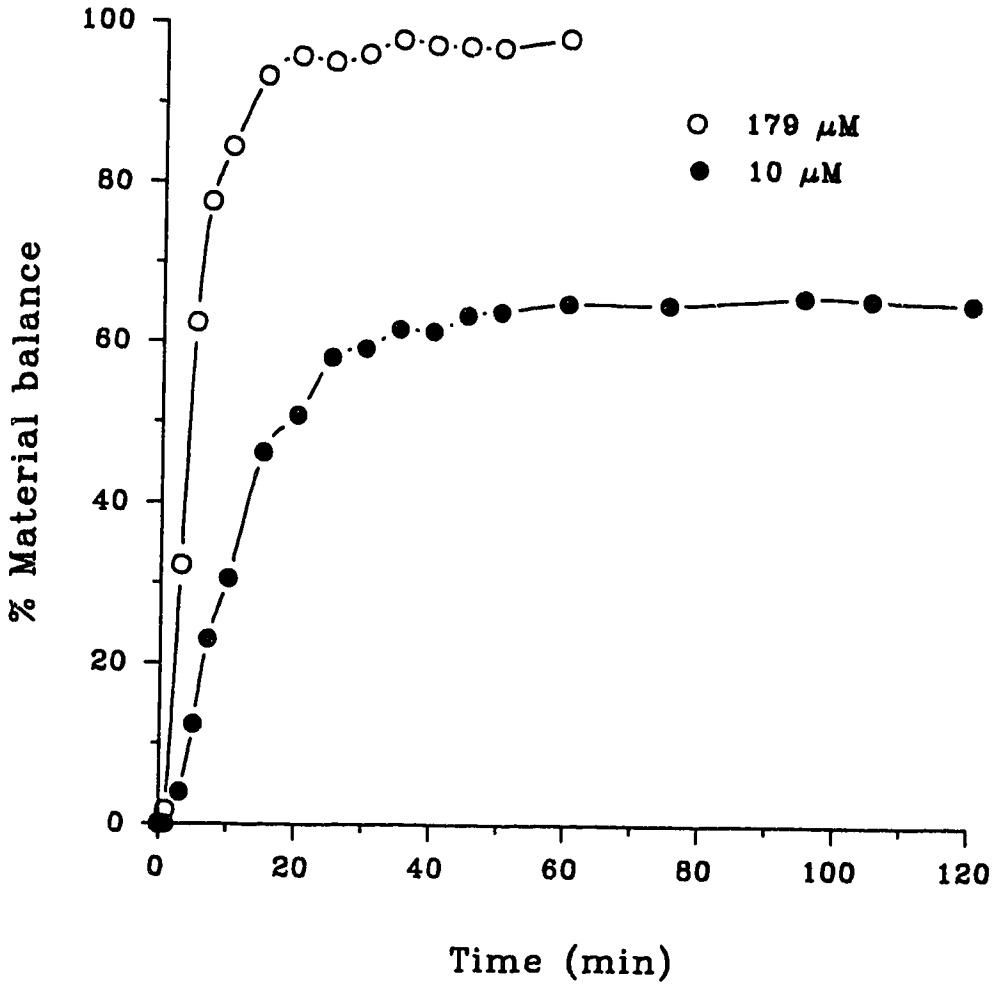

Figure 4.4 Effect of inlet diltiazem (DZ) concentration on total material balance at the effluent with time.

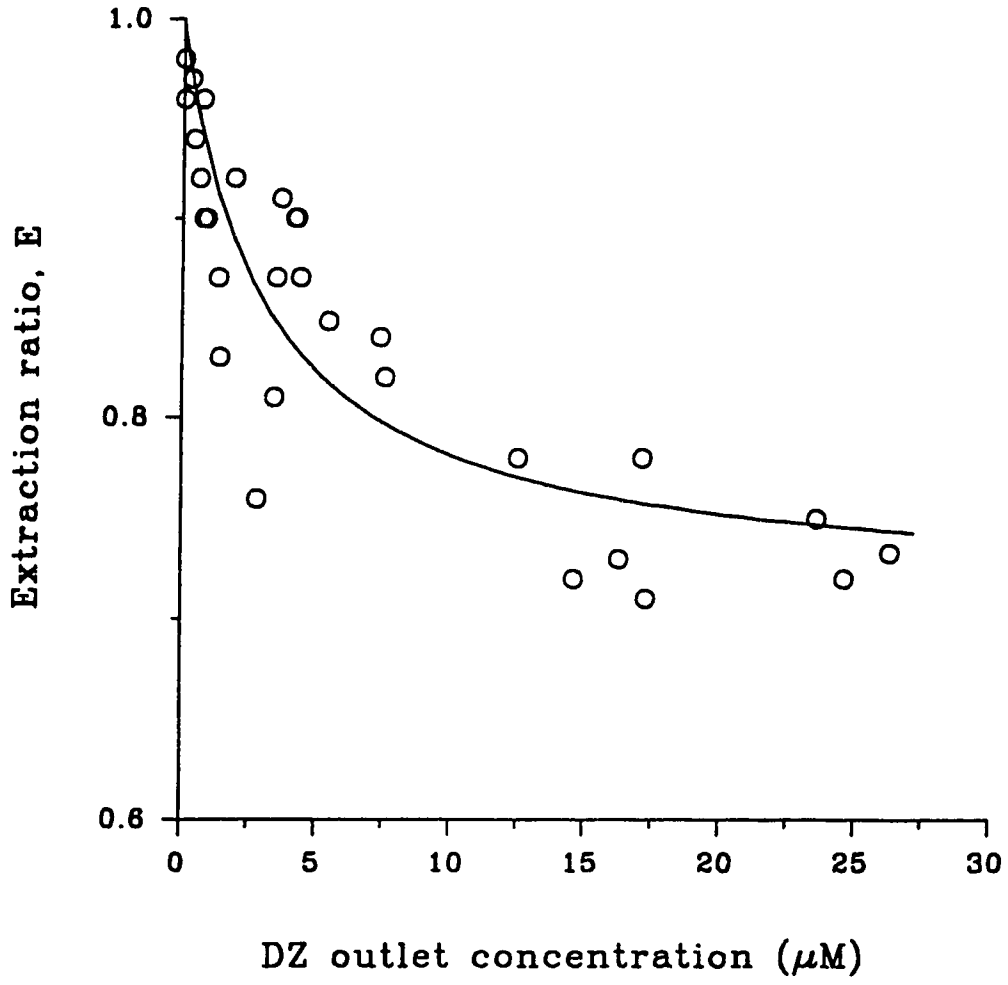

Figure 4.5 Steady state extraction ratio *vs* outlet diltiazem (DZ) concentration.

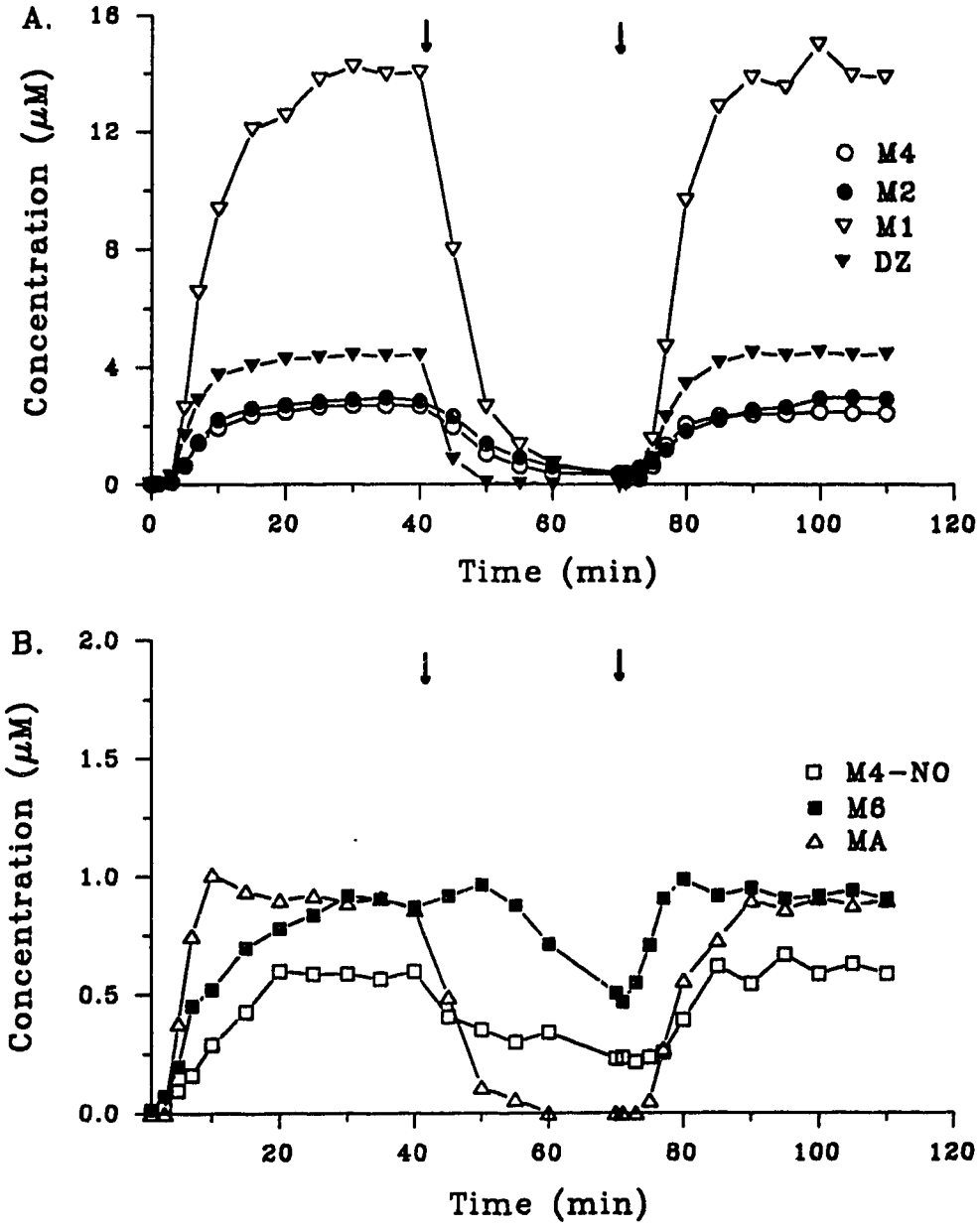

Figure 4.6 Outlet concentration of diltiazem (DZ), M4, M2, M1 (A) and M4-NO, M6, MA (B) *vs* time profiles in a stop-infusion experiment (C_{in} = 34.7 μM). The arrows indicate the cessation and reinitiation of DZ infusion.

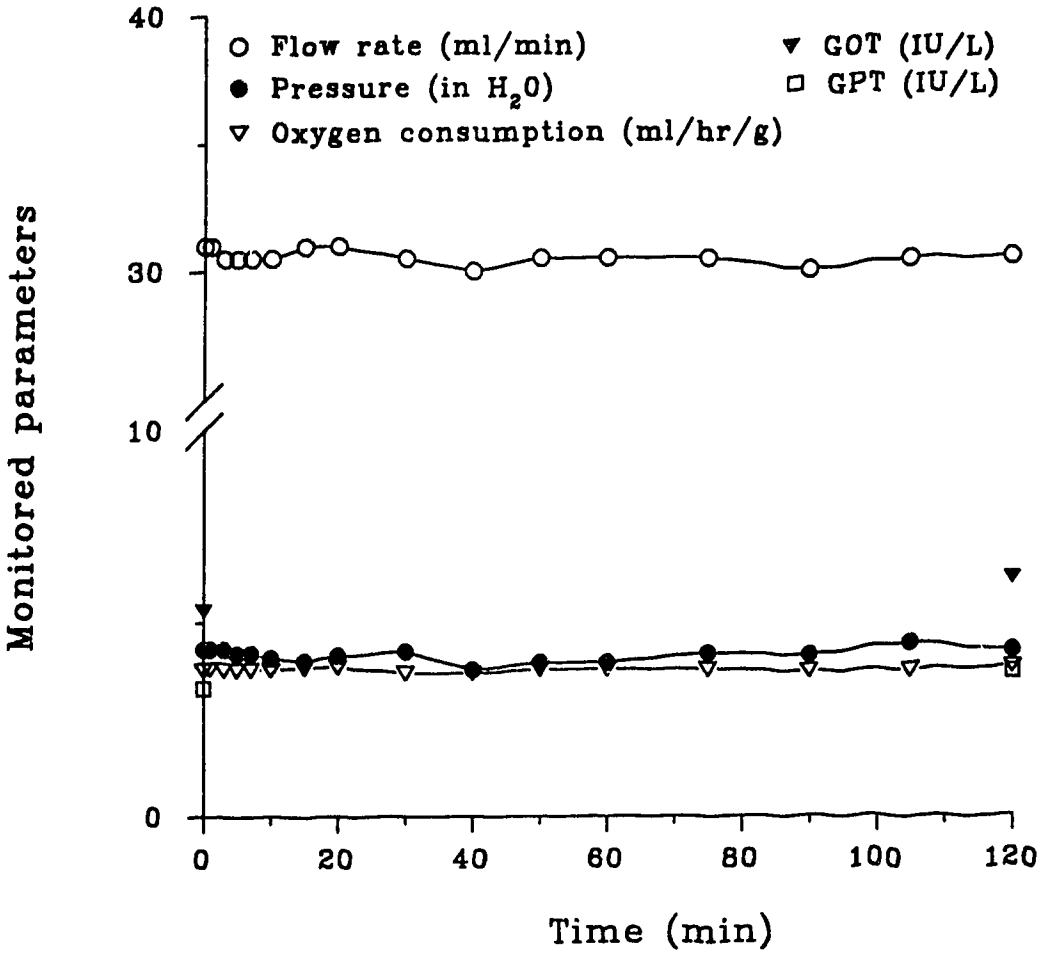

Figure 4.7 A representative set of data from a rat showing the ways in which viability of the isolated liver was monitored. GOT; glutamic oxalacetic transaminase, GPT; glutamic pyruvic transaminase.

4.7 References

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5. KINETIC INTERACTIONS OF LIDOCAINE, DIPHENHYDRAMINE AND VERAPAMIL WITH DILTIAZEM: A STUDY USING ISOLATED PERFUSED RAT LIVER[4]

5.1 Introduction

Various classes of compounds containing a secondary or a tertiary amino-group, such as amphetamines (*Franklin 1977*), SKF 525-A analogs (*Franklin 1977*), erythromycin derivatives (*Larrey et al. 1983*), diphenhydramine analogs (*Bast et al. 1984, 1990*), and tricyclic antidepressants (*Murray and Field 1992*), are capable of inactivating P450 isozymes. The inactivation process involves *N*-dealkylation, which is a common metabolic pathway for alkylamines (*Bast and Noordhoek 1982, Bast et al. 1984, 1990*). Recent investigations in our laboratory have provided evidence that tertiary amines such as lidocaine and diltiazem, are also capable of inactivating *N*-dealkylating isozymes and enzyme inactivation is partially responsible for their time-dependent pharmacokinetics (*Hussain et al. 1992a, Saville et al. 1989*). There is evidence in the literature to suggest that verapamil, a tertiary amine metabolised mainly by *N*-dealkylation (*McIlhenny 1971*), is capable of inactivating enzymes (*Schwartz et al. 1985*). Enzyme inactivation may lead to alteration of pharmacokinetics of other drugs that share the same metabolic pathways. The effect of enzyme inactivation may persist long after the drug responsible for enzyme inactivation is eliminated from the body and may affect

[4] A version of this chapter has been submitted for publication in *Drug Metab Dispos* (1993).

the kinetics of other drugs administered during this period.

Diltiazem, a potent P450 inhibitor (*Renton 1985*), is capable of reducing the clearance of other drugs that share the same elimination pathways. Some of these interactions are clinically important. Interactions of diltiazem have been reported with propranolol (*Dimmitt et al. 1991, Tateishi et al. 1989*), metoprolol (*Tateishi et al. 1989*), digoxin (*North et al. 1986*), cyclosporin (*Brockmoller et al. 1990*), nifedipine (*Frishman et al. 1988*), carbamazepine (*Al-Humayyd 1990*), encanide (*Kazierad et al. 1989*) and imipramine (*Hermann et al. 1992*).

Therapeutically, an antiarrhythmic can be co-administered with a calcium channel blocker for treating serious ventricular arrhythmias (*Fyke et al. 1983, Kapur et al. 1984, Karagueuzian et al. 1985*); therefore, multi-drug therapy involving lidocaine and diltiazem is likely. Diphenhydramine, an over-the-counter antihistamine, is used to treat nausea and vomiting associated with myocardial infarction. Although these tertiary amines are administered together, their mutual interactions have not been evaluated carefully. The present investigation was designed to use a single-pass IPRL model to evaluate interactions between diltiazem and three tertiary amines: lidocaine, diphenhydramine and verapamil. The mode of interaction studied include reversible and irreversible enzyme inhibition.

5.2 Materials and methods

5.2.1 Chemicals and Reagents

The hydrochloride salts of diltiazem and five of its metabolites: M1, M2, M4, M6, M4-NO; and MA fumarate, were generously supplied by Nordic Merrell Dow Research (Laval, Quebec, Canada). Lidocaine and its metabolites, MEGX, 3-OHLID, and GX in the form of hydrochloride salts, were gifts from Astra Pharmaceuticals (Mississauga, Ontario, Canada). Diphenhydramine and its metabolites, *N*-demethyldiphenhydramine (NORDPH) and diphenhydramine *N*-oxide (DPH-NO) in the form of hydrochloride salts, were kindly supplied by Parke Davis (Ann Arbor, MI, USA). All other reagents were of analytical grade.

5.2.2 Animals and Liver Perfusion

Thirty male Sprague-Dawley rats (Biosciences Animal Services, University of Alberta, AB, Canada) weighing 220-250 g were used in the experiments. The animals had free access to rat chow (Wayne Rodent Plox 8604-00, Continental Grain Company, Chicago, IL, USA) and water. The procedure for liver perfusion described by Tam *et al.* (1987) was used with slight modifications. Methoxyflurane (Pitman-Moore, Mississauga, Ontario, Canada) was used to anesthetize the rat. The portal vein was cannulated with a needle catheter (Baxter Healthcare Corporation, Dearfield, IL, USA). The liver was perfused with Krebs-Bicarbonate buffer, equilibrated with 95% O_2/5% CO_2, pH 7.4 at 37°C and at a rate of 3-4 ml/min/g using a peristaltic pump (Ismatec SA,

Cole-Parmer Instrument Company, Niles, IL, USA). The viability of the liver was monitored by evaluating the rate of oxygen consumption measured using a YSI model 6300 oxygen monitor (Yellow Springs Instruments Co. Inc., Yellow Springs, OH, USA); the aspartate and alanine aminotransferase levels in perfusate effluent at the start and at the end of perfusion; perfusion pressure measured by a differential transducer (Model 1151, Rosemount Inc., Minneapolis, MN, USA); concentration of diltiazem and its metabolites in the perfusate effluent, and the physical appearance of the liver. Since diltiazem and its metabolites are susceptible to decomposition at elevated temperature and alkaline conditions (*Caille et al. 1989*), perfusate samples ($\sim$5 ml) were collected on an ice bath and 25 μl of 3N HCl was added to each sample to give a pH $\sim$5.0. Samples were stored at -25°C and were analyzed using HPLC within two weeks of collection. The analyses of lidocaine, diphenhydramine and their metabolites were not subject to this time limitation because they are stable under these conditions.

5.2.3 Co-infusion Study

The objective of this study was to evaluate mutual metabolic inhibition between diltiazem and lidocaine, and diltiazem and diphenhydramine. Rats were randomly divided into two groups (n=5 each). Livers from the rats were perfused initially with diltiazem alone (inlet concentration, C_{in} = 40.6 $\pm$ 5.6 μM) for a period of 40 min followed by a co-infusion with either lidocaine (C_{in} = 42.7 $\pm$ 4.0 μM) or diphenhydramine (C_{in} = 43.6 $\pm$ 0.6 μM) for another 40 min. After the initiation of diltiazem infusion, effluent samples were collected at 0, 1, 3, 7, 10 min, every 5 min up

to 40 min, and at 41, 43, 45, 47, 50 min and every 5 min thereafter up to 80 min for the measurement of outlet concentration (C_{out}). Inlet samples were collected at 0, 2, 12, 32, 42, 51, 64 and 79 min for the determination of C_{in} of diltiazem.

5.2.4 Pretreatment Study

The goal of this study was to investigate the effect of *in-vivo* enzyme inactivation by tertiary amines on the kinetics of diltiazem. Rats were randomly divided into five groups (n=4 each). Each group was treated either with diltiazem (20 mg/kg, DZ group) or lidocaine (30 mg/kg, LID group) or diphenhydramine (20 mg/kg, DPH group) or verapamil (10 mg/kg, VP group) or saline (0.4 ml, control group) intraperitoneally (i.p.) for 3-5 days. These doses were chosen because they were reported to provide therapeutic concentrations (*Coutts et al. 1987, Drach et al. 1970, Keenaghan and Boyes 1972, Nakamura et al. 1987, Saville 1989, Yeung et al. 1990*). The liver was harvested and perfused as described earlier on the following day; diltiazem was infused for 100 min. Effluent samples from the liver were collected at 0, 1, 3, 5, 7, 10 min, every 5 min up to 50 min and every 10 min thereafter up to 100 min. Inlet samples were collected at 0, 2, 6, 12, 24, 51, 81 and 98 min.

5.2.5 HPLC Analysis

Outlet concentrations of diltiazem, lidocaine, diphenhydramine and their metabolites in the liver perfusate were determined using a reversed phase HPLC procedure. The HPLC system (Waters Instruments, Mississauga, Ontario, Canada)

consisted of a Model 501 pump, a Model 710B automatic injector and a Model 441 UV detector set at 214 nm. Separation of individual components was achieved using a Waters 4 μm C_{18} Novapak cartridge column (10 cm x 8 mm I.D.). The mobile phase was an aqueous solution of 0.092% H_3PO_4 and 0.2% triethylamine (74 parts, v/v) and acetonitrile (26 parts, v/v), and the flow rate was 2 ml/min. Bupivacaine HCl (Sigma Chemicals Co., St Louis, MO, USA) was used as the internal standard in all studies except that when samples from diltiazem and diphenhydramine co-infusion study were analyzed; in that study lidocaine HCl was used as the internal standard. Calibration curves were linear over the concentration range studied (10-2000 ng/ml). The intra- and inter-day coefficients of variation for the analysis of diltiazem and its metabolites were less than 10%.

5.2.6 Pharmacokinetic Analysis

Steady state concentration (C_{ss}) and time to reach steady state T_{ss} were statistically determined according to the procedure described by Saville *et al.* (*1989*). Steady state extraction ratio (E), hepatic drug clearance Cl_H (ml/min/g liver) and material balance (MB) were calculated using the following equations:

$$E = \frac{C_{in} - C_{out}}{C_{in}} \qquad (1)$$

$$MB = \frac{C_{known} \times 100}{C_{in}} \qquad (3)$$

$$Cl_H = Q \times E \qquad (2)$$

where, Q is the perfusion flow rate (ml/min) and C_{known} (μM) is the total concentration of diltiazem and its known metabolites in the effluent at steady state. The total displacement of diltiazem and its metabolites from the liver tissue binding sites during

$$Total\ displacement = \int_{T_{co}}^{T_{ss}} Q(C_{out} - C_{nss})dt \qquad (4)$$

the co-infusion study was calculated using the following equation:

where, T_{co} represents the time when $C_{out} \geq C_{nss}$ after lidocaine or diphenhydramine infusion; C_{nss} being the second steady state concentration. The computer program LAGRAN (*Rocci and Jusko 1983*) was used to calculate the displacement. The inhibition constant, K_i (μM) for diltiazem metabolism by lidocaine or diphenhydramine during the co-infusion study was calculated using the following equations:

$$V = \frac{V_{max} S}{S + K_m} \qquad (5)$$

$$V_i = \frac{V_{max} S_i}{S_i + K_m\left(1 + \frac{I}{K_i}\right)} \qquad (6)$$

where V and V_i are steady state rate of diltiazem metabolism (nmol/min/g liver) in the absence and in the presence of lidocaine or diphenhydramine, S and S_i are C_{ss} of diltiazem (μM) during diltiazem infusion and co-infusion with lidocaine or

diphenhydramine, K_m is the Michaelis Menten constant for diltiazem metabolism (μM), V_{max} is the maximum rate of diltiazem metabolism (nmol/min/g liver) at steady state, I is the C_{ss} of lidocaine or diphenhydramine (μM). K_i can be solved by dividing equation (5) by equation (6):

$$K_i = \frac{K_m I V_i S}{S S_i (V - V_i) + K_m (V S_i - V_i S)} \qquad (7)$$

The inhibition constant K_i for diltiazem metabolism by lidocaine or diphenhydramine was assumed to be due to competitive inhibition only. Outlet concentrations from the liver were assumed to be in equilibrium with the concentration within the liver (well mixed model) (*Rowland et al. 1973*). From a previous study (*Hussain et al. 1992a*), the calculated K_m values for diltiazem metabolism *via* known and unknown pathways are 17.13 and 4.76 μM, respectively. The steady state rate of diltiazem metabolism by known pathways (V_{known}) was calculated using the following equation:

$$V_{known} = \sum V_j \qquad (8)$$

where j=1 to 6 for the six measured metabolites. The equation for the steady state rate of diltiazem metabolism by unknown pathways ($V_{unknown}$) is as follows:

$$V_{unknown} = V_{total} - V_{known} \qquad (9)$$

where V_{total} is the total rate of diltiazem metabolism at steady state.

5.2.7 Statistics

All data are reported as mean ± SD. One way analysis of variance (ANOVA) was used to evaluate differences in kinetic parameters between the groups in the pretreatment studies. If a difference was detected, Duncan's multiple range test was used for multiple comparison of means. Paired t-test was used to detect differences between the kinetic parameters of diltiazem in the presence and absence of lidocaine or diphenhydramine co-infusion. In each individual rat, the 95% confidence interval (*Saville et al. 1989*) test was used to evaluate differences between the two steady state levels of the corresponding diltiazem and its metabolites during the co-infusion studies. If the points in the C_{ss_2} do not fall within the bounds of the first C_{ss_1}, the C_{ss_2} was considered as significantly different. Unpaired t-test was used to detect differences between the K_i's and the total displacements of diltiazem by lidocaine and diphenhydramine. The level of significance was set at $p=0.05$.

5.3 Results

Co-infusion with lidocaine at the steady state of diltiazem caused an abrupt increase in the C_{out} of diltiazem which then declined to a new C_{ss} that was higher than the initial C_{ss} level (Figure 5.1). The concentration *vs* time profiles of diltiazem metabolites M1 and M2 paralleled that of diltiazem. The overshoot in the diltiazem concentration, which occurred within 3-10 min, was probably due to a displacement of diltiazem from the tissue binding sites in the liver. In a pilot study, it has been shown that diltiazem does not adsorb on to the tubing used in the perfusion system. Thus, the

sharp increase in the diltiazem outlet concentration, induced by lidocaine or diphenhydramine, was not due to an artifact which could occur if lidocaine displaced the adsorbed diltiazem on the tubing. The total displacement of diltiazem due to lidocaine infusion was 111.5 ± 132.5 nmol/g of liver.

The steady state concentration of diltiazem was significantly increased (mean = 46%) during lidocaine infusion (Figure 5.1 and Table 5.1). This increase was accompanied by an increase in the C_{ss} values of the primary metabolites M1 and MA (mean = 17 and 23%, respectively) and a decrease in the C_{ss} of the *O*-demethylated metabolites M4 and M6 (mean = 18 and 24%, respectively). The mean C_{ss} values of M2 were significantly increased in three rats and that of M6 and M4-NO were significantly decreased in four and three rats, respectively. Once lidocaine infusion was stopped the perturbations produced by lidocaine disappeared (Figure 5.1).

Lidocaine co-infusion significantly decreased the E and Cl_H values of diltiazem (Table 5.2). It took approximately 19 min for diltiazem to achieve the new steady state, probably due to the perturbation in the tissue binding and metabolism of diltiazem in the liver produced by lidocaine infusion.

The characteristic rise to a maximum in the concentration versus time profile of the *N*-deethylated metabolite of lidocaine, MEGX, which was observed when lidocaine was infused alone in IPRL (*Tam et al. 1987*) was absent in this co-infusion study (Figure 5.2). The lidocaine level came to a steady state quickly and the E values for lidocaine were lower than that reported in the literature (0.5-0.7 *vs* 0.3-0.5) (*Saville et al. 1989,*

Tam et al. 1987). The C_{ss} of MEGX and 3-OHLID were 5.2 ± 1.0 and 1.5 ± 0.2 μM, respectively at the C_{ss} of lidocaine (25.2 ± 3.4 μM). These values are lower than those reported by Tam *et al.* (*1987*) at similar C_{ss} of lidocaine. These observations suggest that diltiazem inhibits both the *N*-dealkylation and aromatic hydroxylation of lidocaine.

Diphenhydramine co-infusion also resulted in a rapid increase in the effluent diltiazem level which was followed by a decline to a new and higher C_{ss} (Figure 5.3). The spike in the outlet concentration *vs* time profile of diltiazem occurred within 3-7 min. The total amount of diltiazem displaced by diphenhydramine was 47.0 ± 26.4 nmol/g of liver.

Diphenhydramine co-infusion caused a significant increase in the C_{ss} level of diltiazem (mean = 45%, Table 5.3). Steady state concentrations of M1, MA and M2 were significantly increased (mean = 54, 51, 34% respectively) while M4, M6 and M4-NO levels were significantly decreased (mean = 63, 58, 77% respectively) (Table 5.3). The concentration versus time profiles of diphenhydramine and two of its metabolites collected from a rat are shown in Figure 5.4.

The co-infusion with lidocaine or diphenhydramine resulted in a significant increase in the material balance of diltiazem (Tables 5.2 and 5.4). The total C_{ss} levels of known diltiazem metabolites were increased (25.1 ± 4.2 and 22.1 ± 1.6 *vs* 26.3 ± 4.3 and 26.4 ± 1.5 μM, respectively) and the total C_{ss} levels of unknown diltiazem metabolites were decreased (14.9 ± 5.01 and 12.2 ± 2.5 *vs* 12.0 ± 4.9 and 7.5 ± 1.4 μM, respectively). K_i values for the unknown pathways of diltiazem metabolism were

calculated for lidocaine and diphenhydramine; these values were 27.3 ± 16.6 and 11.1 ± 6.0 μM, respectively and were not significantly different (p=0.109).

The effect of drug pretreatment on diltiazem kinetics is presented in Table 5.5. Pretreatment with any one of the four drugs, diltiazem, lidocaine, diphenhydramine and verapamil, did not change the C_{ss}, E or Cl_H values of diltiazem when compared to controls. However, diltiazem and lidocaine pretreatment caused a significant increase in the total material balance. In groups pretreated with diltiazem and lidocaine, there was a trend towards increases in the recoveries of M1, M2, M4 and M6 (Table 5.6). In the control group, the concentration time profile of MA (a minor metabolite), in two rats showed a characteristic maximum before reaching a steady state. This phenomenon was not observed in the rats of the drug pretreated groups. These results are consistent with the observation that the pretreatment with these drugs caused enzyme inactivation.

Two rats, one from the diphenhydramine co-infusion group and one from the lidocaine pretreatment group were excluded from the study because their livers were not viable throughout the study.

5.4 Discussion

The abrupt increase in the diltiazem level (Figures 5.1 and 5.3) during the co-infusion studies may be explained by a reduction in the binding and or the extent of distribution of diltiazem in the rat liver. In a previous study using IPRL, we have shown that the time-dependent kinetics of diltiazem were mainly due to reversible tissue binding (*Hussain et al. 1992a*). Binding of lidocaine to liver tissues was also partially

responsible for the time-dependent kinetics of lidocaine (*Saville et al. 1989, Tam et al. 1987*). Preliminary studies of diphenhydramine using IPRL (n=4, C_{in} 12.2-79.0 μM) also suggest that the relatively long time to achieve a steady state (25-50 min) may be at least partially due to the binding of diphenhydramine in the rat liver. Binding to liver tissues was also observed for imipramine, a tertiary amine, in IPRL (*Erland and Gram 1982*). These basic lipophilic drugs have a high affinity for the cellular region of the liver; therefore the liver acts as a large reservoir. Our data indicate that lidocaine displaces some diltiazem from its binding sites in liver tissues (Figure 5.1). Similarly, the long T_{ss} of diphenhydramine (Table 5.4 and Figure 5.4) during the co-infusion studies could be partially due to tissue binding which resulted in the displacement of diltiazem from its binding sites. It is likely that lidocaine and diphenhydramine and/or their metabolites partially compete for the same tissue binding sites of diltiazem in the rat liver.

The amounts of diltiazem displaced by lidocaine and diphenhydramine were not significantly different (111.5 $\pm$ 132.5 *vs* 47.0 $\pm$ 26.4 nmol/g liver, p=0.375) and were small compared to the total capacity of diltiazem binding which was 669.5 $\pm$ 156.5 nmol/g liver at a C_{in} of 35.5 $\pm$ 3.2 μM of diltiazem (*Hussain et al.1992a*). Partitioning of diltiazem in the liver tissue *vs* buffer solution in the sinusoid was higher than that of lidocaine (8.9 $\pm$ 0.8 *vs* 5.0 $\pm$ 1.3) (*Hussain et al.1992a*). These values indicate that at steady state, concentrations of diltiazem and lidocaine in the liver tissue are approximately nine and five times higher than those in the sinusoid and suggest a higher affinity of diltiazem for the binding sites in the liver. This observation may explain the

small displacement of diltiazem by lidocaine from its binding sites. Similarly, diltiazem had a higher affinity for binding to liver tissue than diphenhydramine and thus diphenhydramine infusion induced only a small change in the binding of diltiazem. Another possibility may be that diltiazem also binds to different sites in the liver that do not bind lidocaine and diphenhydramine.

The concentration profile of MEGX (Figure 5.2) resembled that of the second lidocaine infusion results reported by Saville *et al.* (*1989*). In their stop-flow experiment (initial infusion followed by a second infusion separated by a washout period), the characteristic maximum in the concentration *vs* time profile of MEGX was absent during the second infusion and the T_{ss} for lidocaine still exceeded the anticipated time of 3-5 min as indicated by the mean residence time of the perfusate in the liver. These observations suggest that 1) diltiazem caused the inactivation of at least one isozyme that is partially responsible for the *N*-dealkylation of lidocaine and 2) reversible binding and/or distribution of lidocaine to liver tissues is mainly responsible for the delay in the attainment of steady state concentration when enzyme activities were constant.

Co-infusion of diltiazem and lidocaine induced a significant increase in the steady state concentration of diltiazem (Table 5.1). This change was accompanied by a significant increase in the formation of the primary *N*-demethyl (MA) and deacetyl (M1) metabolites, and a significant decrease in secondary (M4) and tertiary (M6) *O*-dealkylated metabolite concentrations. Metabolite to parent drug ratios for the two primary metabolites MA and M1 were not significantly changed during the co-infusion. These observations, coupled with the increase in the material balance of diltiazem (Table 5.2),

suggest that the increase in the C_{ss} of diltiazem was due to an inhibition of other unidentified primary pathways of diltiazem metabolism. Recently, Li *et al.* (*1992*) have reported two new primary diltiazem metabolites identified as the *O*-demethyldiltiazem and diltiazem *N*-oxide. Since lidocaine depresses the levels of M4, M6 and M4-NO (Figure 5.1 and Table 5.1) which are formed by the *O*-demethylation and *N*-oxidation pathways, it is conceivable that the newly reported pathways (28) would also be inhibited. In addition to the four pathways (deacetylation, *N*-demethylation, *O*-demethylation and *N*-oxidation) that we quantified, oxidative deamination and aromatic hydroxylation of diltiazem by P450 have also been reported in rats (*Meshi et al. 1971, Nakamura et al. 1990, Sugawara et al. 1988*). Lidocaine is also known to inactivate P450 isozyme(s) involved in aromatic hydroxylation (*Masubuchi et al. 1992*), therefore, it is not surprising that some of these unquantified pathways are inhibited.

The metabolite to drug ratios for primary metabolites MA and M1 were not significantly changed during the diphenhydramine co-infusion. Material balance of diltiazem increased significantly during the co-administration of diphenhydramine (Table 5.4). These results suggest that co-infusion of diphenhydramine caused an inhibition of *O*-dealkylation, *N*-oxidation and other unidentified metabolic pathways of diltiazem in the rat liver. Although the K_i of diphenhydramine for unknown metabolic pathways of diltiazem had a tendency towards lower values than that of lidocaine (11.1 $\pm$ 6.0 *vs* 27.3 $\pm$ 16.6 μM, respectively), they were not significantly different ($p=0.109$). This is probably due to large interindividual differences. The K_i values suggest that diphenhydramine can have a higher affinity than lidocaine for the unknown metabolic

pathways of diltiazem, and that diphenhydramine is more potent in inhibiting the unknown metabolic pathways of diltiazem when compared to lidocaine.

In a preliminary study on diphenhydramine (C_{in} 12.2-79.0 μM), the E values of diphenhydramine were found to range from 0.44 to 0.85. These values are higher than those obtained when diphenhydramine was co-infused with diltiazem (Table 5.4), suggesting that diltiazem inhibits the metabolism of diphenhydramine in IPRL. The C_{ss} of NORDPH and DPH-NO were 7.5 $\pm$ 0.5 and 4.3 $\pm$ 0.5 μM at the diphenhydramine C_{ss} of 18.0 $\pm$ 1.4 μM. The C_{ss} of NORDPH is lower than that obtained at a comparable C_{ss} of diphenhydramine in the preliminary study (10.1-15.3 μM at C_{ss} of diphenhydramine 19.5 μM). This observation suggests that diltiazem causes an inhibition in the *N*-demethylation of diphenhydramine.

Enzyme inactivation induced by tertiary amines did not change the kinetics of diltiazem (Table 5.5) presumably because *N*-demethylation of diltiazem is a minor metabolic pathway in the rat. In the human, *N*-demethylation of diltiazem is one of the major elimination pathways (*Hussain et al. 1992b, Yeung et al. 1990*) and pretreatment with tertiary amine drugs may change the human kinetics of diltiazem. However, pretreatment with diltiazem or lidocaine in the rat caused a significant increase in the material balance. The increase in the material balance was due to a tendency towards increased recoveries of metabolites M1, M2, M4 and M6. Since these metabolites can undergo further metabolism by conjugation or other unmeasured pathways, it is possible that pretreatment with these two tertiary amines inhibits their secondary metabolism.

The results of this study indicate that lidocaine or diphenhydramine interacts with diltiazem when given together. The observed interactions are due to a displacement of diltiazem from its binding sites in the liver and inhibition of diltiazem metabolism and thus both drugs have the potential to influence the *in vivo* kinetics of diltiazem. Inhibitory effects of diltiazem on lidocaine and diphenhydramine metabolism are also evident from this study. Enzyme inactivation induced by a pretreatment with diltiazem, lidocaine, diphenhydramine or verapamil did not affect the steady state level of diltiazem and the primary metabolites in the rat. However, pretreatment with lidocaine and diltiazem affects the secondary metabolic pathways of diltiazem resulting in an increased material balance.

5.5 Tables

Table 5.1 Steady state concentrations (μM) of diltiazem and its metabolites (mean ± SD) during the co-infusion study with lidocaine (n=5).

Compounds	Diltiazem infusion	Lidocaine co-infusion
Diltiazem	4.58 ± 1.18	6.37 ± 1.22*
MA	1.09 ± 0.20	1.33 ± 0.21*
M1	13.60 ± 3.07	15.83 ± 3.22*
M2	4.02 ± 1.15	4.27 ± 1.23[a]
M4	3.27 ± 0.27	2.68 ± 0.32*
M6	1.43 ± 0.26	1.08 ± 0.18[b]
M4-NO	1.69 ± 1.25	1.13 ± 0.61[a]

*significantly different in all rats.
[a]significantly different in 3 rats.
[b]significantly different in 4 rats.

Table 5.2 Steady state kinetic parameters of diltiazem and lidocaine (mean ± SD) during the co-infusion study with lidocaine (n=5).

Kinetic parameters	Diltiazem infusion	Co-infusion with lidocaine	
	Diltiazem	Diltiazem	Lidocaine
C_{ss} (μM)	44.59 ± 8.47	45.42 ± 7.56	42.73 ± 4.02
E	0.90 ± 0.03	0.86 ± 0.04*[a]	0.41 ± 0.08
Cl_H (ml/min/g liver)	2.84 ± 0.16	2.72 ± 0.19*[b]	1.28 ± 0.21
T_{ss} (min)	25.00 ± 6.12	19.2 ± 7.46	12.60 ± 3.91
MB (%)	66.99 ± 5.23	72.44 ± 5.39*	74.66 ± 10.44

*significantly different from the values obtained from diltiazem infusion.
a, p=0.049; b, p=0.047

Table 5.3 Steady state concentrations (μM) of diltiazem and its metabolites (mean ± SD) during the co-infusion study with diphenhydramine (n=4).

Compounds	Diltiazem infusion	Diphenhydramine co-infusion
Diltiazem	3.70 ± 1.89	4.94 ± 1.56*
MA	0.61 ± 0.12	0.92 ± 0.24*
M1	12.08 ± 1.63	18.53 ± 1.37*
M2	3.88 ± 0.92	5.11 ± 0.93*
M4	2.63 ± 0.30	0.96 ± 0.04*
M6	1.18 ± 0.31	0.48 ± 0.08*
M4-NO	1.67 ± 0.57	0.38 ± 0.09*

*significantly different in all rats.

Table 5.4 Steady state kinetic parameters of diltiazem and diphenhydramine (mean ± SD) during the co-infusion study with diphenhydramine (n=4).

Kinetic parameters	Diltiazem infusion	Co-infusion with diphenhydramine	
	Diltiazem	Diltiazem	Diphenhydramine
C_{ss} (μM)	37.93 ± 2.33	38.79 ± 1.95	43.63 ± 0.59
E	0.90 ± 0.06	0.87 ± 0.04	0.32 ± 0.02
Cl_H (ml/min/g liver)	2.68 ± 0.17	2.60 ± 0.12	0.94 ± 0.05
T_{ss} (min)	26.25 ± 2.50	22.5 ± 2.89	23.75 ± 4.79
MB (%)	68.10 ± 5.35	80.84 ± 2.89*	68.51 ± 1.85

*significantly different from the values during initial diltiazem infusion.

Table 5.5 Steady state kinetic parameters of diltiazem (mean ± SD) in the pre-treatment study with tertiary amines (n=4).

Kinetic parameter	Groups				
	Control	DZ	LID[a]	DPH	VP
C_{in} (μM)	14.86 ± 1.63	14.62 ± 3.33	14.25 ± 2.56	15.83 ± 0.44	16.50 ± 0.23
E	0.93 ± 0.02	0.93 ± 0.01	0.91 ± 0.02	0.95 ± 0.01	0.93 ± 0.03
Cl_H (ml/min/g liver)	2.97 ± 0.08	2.86 ± 0.15	2.84 ± 0.19	3.03 ± 0.22	2.99 ± 0.13
MB (%)	65.85 ± 6.93	88.95 ± 8.86[b]	88.37 ± 19.37[b]	67.18 ± 9.23	65.61 ± 9.11
T_{ss} (min)	41.25 ± 6.29	42.5 ± 5.00	46.67 ± 5.77	32.50 ± 6.45[c]	37.50 ± 5.00

[a]n=3.
[b]values significantly different from that of control, DPH and VP groups.
[c]values significantly different from that of LID group.

Table 5.6 Recoveries (% of dose) of diltiazem and its metabolites (mean ± SD) in effluent perfusate samples at steady state in the pre-treatment groups (n=4)[a].

Compounds	Pretreatment group				
	Control	DZ	LID[b]	DPH	VP
Diltiazem	7.57 ± 2.29	6.66 ± 0.95	8.85 ± 1.69	4.86 ± 1.21	6.92 ± 3.00
MA	1.72 ± 0.45	1.62 ± 0.44	1.84 ± 0.54	1.39 ± 0.23	1.89 ± 0.83
M1	22.07 ± 4.49	27.59 ± 5.07	26.89 ± 3.06	22.23 ± 4.19	24.06 ± 0.77
M2	7.56 ± 0.50	13.48 ± 2.09	13.36 ± 6.44	11.48 ± 2.38	11.21 ± 2.54
M4	14.44 ± 2.98	19.48 ± 1.03[c]	20.19 ± 7.92[c]	12.67 ± 1.86	10.72 ± 3.84
M6	8.85 ± 1.89	12.51 ± 0.75	12.68 ± 5.55	8.27 ± 2.60	7.65 ± 2.85
M4-NO	3.64 ± 0.81	7.64 ± 0.09[d]	3.64 ± 0.92	6.28 ± 3.78	3.21 ± 2.02

[a]overall recovery calculated as MB (%) is given in Table 5.5.
[b]n=3.
[c]values significantly different from that of DPH and VP groups.
[d]values significantly different from that of control, LID and VP groups.

5.6 Figures

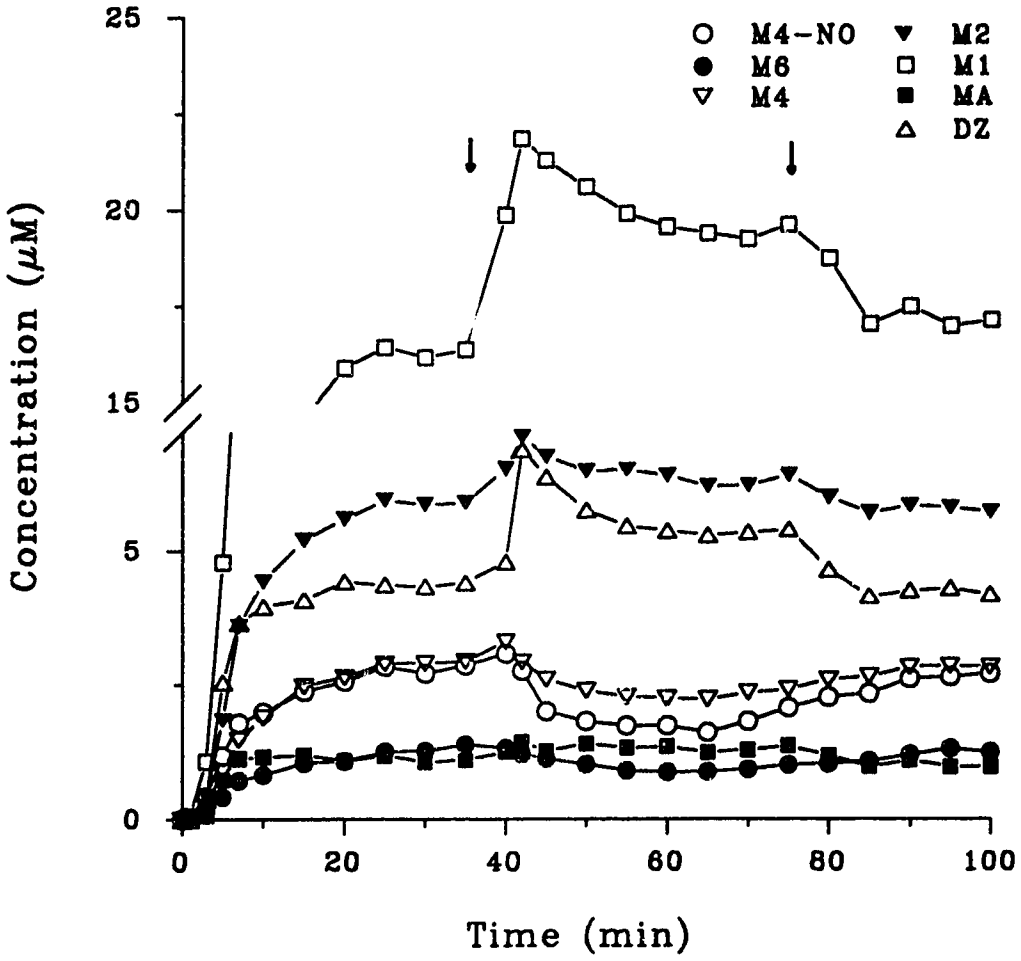

Figure 5.1 Concentration versus time profiles of diltiazem (DZ) (C_{in} = 57.6 μM) and its metabolites during co-infusion with lidocaine (C_{in} = 39.5 μM). Arrows show initiation and cessation of lidocaine infusion.

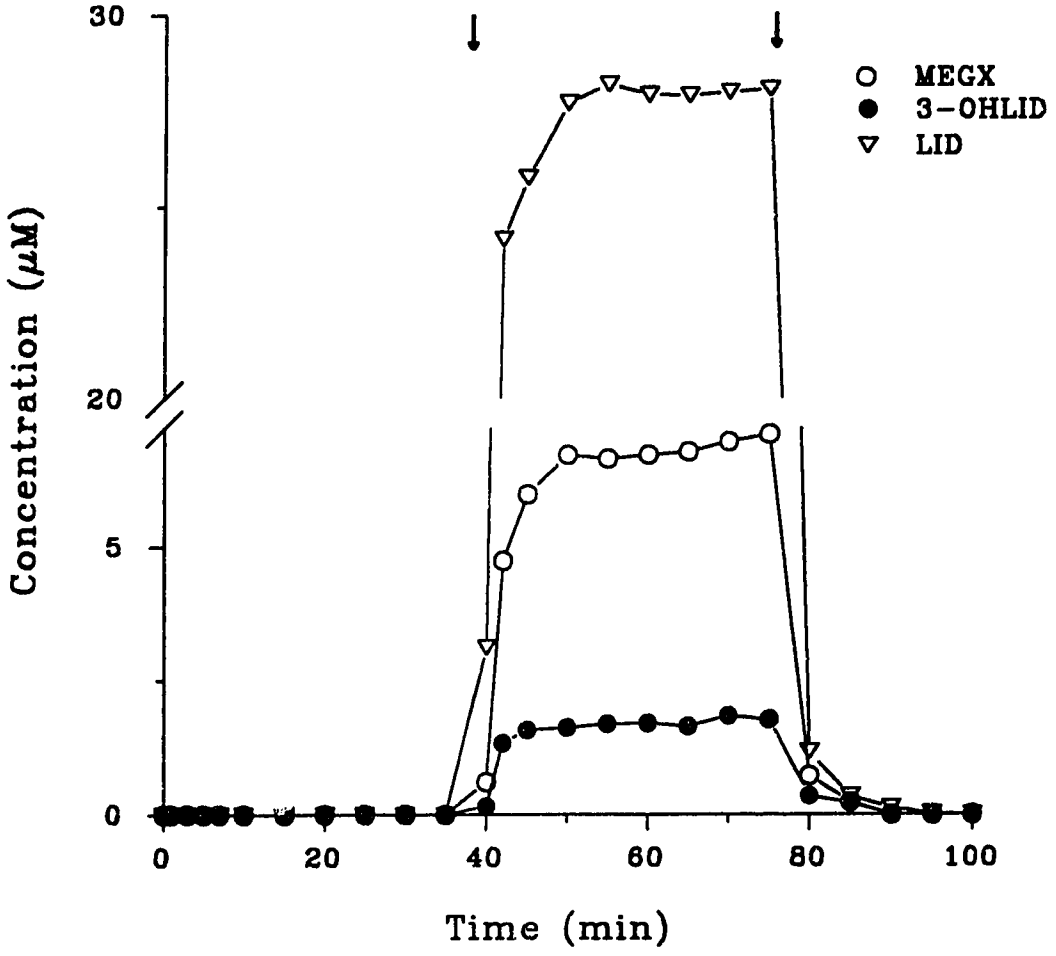

Figure 5. 2 Concentration versus time profiles of lidocaine (LID) (C_{in} = 39.5 μM) and its metabolites during co-infusion with diltiazem (C_{in} = 57.6 μM). Arrows show initiation and cessation of LID infusion.

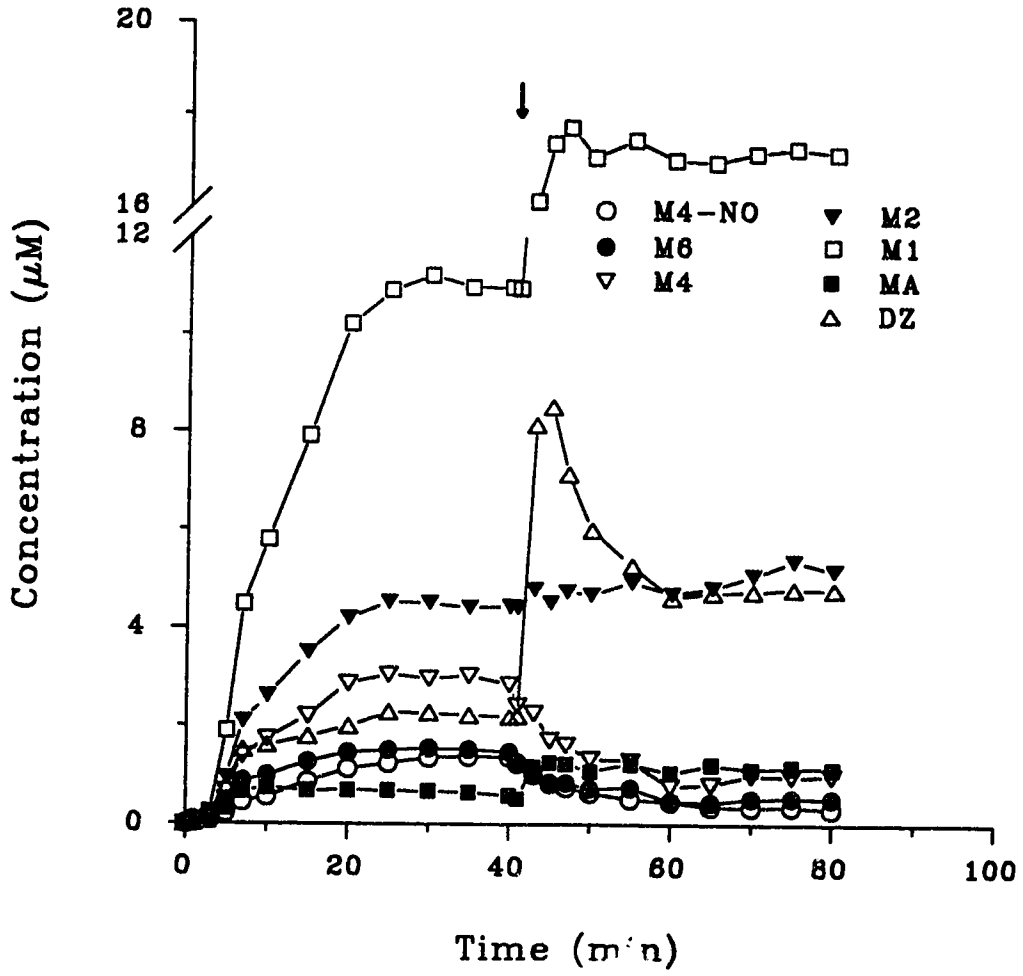

Figure 5. 3 Concentration versus time profile of diltiazem (DZ) (C_{in} = 38.7 μM) and its metabolites during co-infusion with diphenhydramine. Arrow show initiation of diphenhydramine infusion (C_{in} = 43.9 μM).

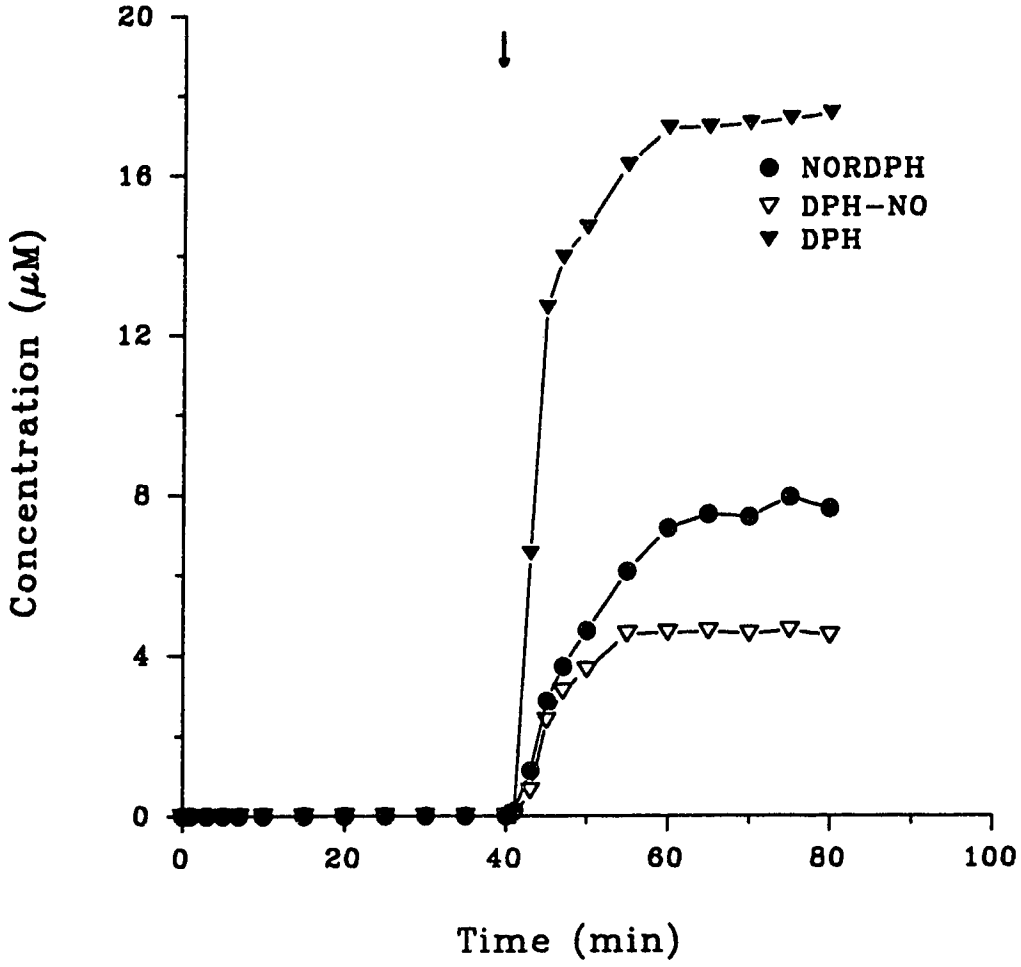

Figure 5.4 Concentration versus time profiles of diphenhydramine (DPH) (C_{in} = 43.9 μM) and its metabolites during co-infusion with diltiazem (C_{in} = 38.7 μM). Arrow shows initiation of DPH infusion.

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6. SUMMARY AND CONCLUSIONS

Diltiazem, a calcium channel blocking agent, is used in treating a number of cardiovascular diseases such as angina pectoris, hypertension and arrhythmias (*Zelis 1982, Inouye et al. 1984, Akhtar et al. 1989*). This drug has a protective effect on ischemic myocardium and reduces arrhythmias associated with the ischemia (*Clozel et al. 1983, Flaim and Zelis 1981, Godet et al. 1987*). Because of these properties, diltiazem is used in patients who require coronary artery bypass surgery. Diltiazem is rapidly cleared from the body *via* hepatic metabolism and it is subjected to significant first-pass elimination (*Buckley et al. 1990*). The drug is relatively highly plasma protein bound. Thus, conditions or drugs that can alter hepatic blood flow, enzyme activities, and plasma and/or tissue protein binding have the potential to change the pharmacokinetics of diltiazem. The objectives of this study were to evaluate the relative importance of these physiological parameters in determining diltiazem kinetics.

The CPB procedure used during cardiac surgery induces profound physiological changes and has the potential to alter the kinetics of drugs (*Buylaert et al. 1989*). Since a therapeutic concentration of diltiazem is necessary to diminish the coronary artery vasospasm (*Buffington et al. 1981, Buxton et al. 1981*) and calcium-induced damage during the reperfusion (*Braunwald and Kloner 1985*), both associated with the cardiac surgery, it is imperative to know the fate of diltiazem in these patients during and immediately after surgery. Prior to this investigation, no study had been performed to evaluate the effect of anesthesia and CPB on the pharmacokinetics of diltiazem.

Therefore, the goals were to (1) characterize the pharmacokinetics of preoperative oral diltiazem in patients undergoing coronary artery bypass grafting and (2) to evaluate whether therapeutic levels of unbound diltiazem are present during and immediately after CPB. Before the proposed studies, we developed a new HPLC assay method for simultaneous separation and quantification of diltiazem and six of its metabolites in plasma. Previous assay methods either could not measure all known metabolites of diltiazem or were tedious, less sensitive and non-specific. The HPLC method developed by us is simple, sensitive and specific. The lower limit of quantification of the assay is 5 ng/ml for diltiazem and its metabolites. This method is also reproducible; inter-day and intra-day coefficients of variation are less than 10%. The applicability of the method is shown by determining the kinetics of diltiazem and its metabolites in patients receiving chronic diltiazem therapy. MA, M1 and M2 were found to be the major metabolites in patients who required bypass surgery.

The pharmacokinetics of oral diltiazem in patients who required bypass surgery were studied on the day before and on the day of surgery. Pharmacokinetic parameters measured on the day before were comparable to those reported in the literature (*Smith et al. 1983*). The oral clearance was reduced with the higher dose on the day before surgery. Deacetylation and *N*-demethylation are the two major metabolic pathways of diltiazem in the humans. These metabolic pathways were not saturated in patients receiving a dose of diltiazem and thus the saturation of metabolism does not seems to play a significant role in the observed reduction of oral clearance. This observation, however is consistent with saturable tissue binding. Results obtained from IPRL support

this hypothesis; hepatic tissue binding plays a dominant role in causing the time-dependent reduction in diltiazem clearance and this binding is saturable. Saturable tissue binding can also be used to explain the observation that patients on higher chronic dosages of diltiazem had lower oral clearance values. The lower clearance is not reflected in the measured metabolite levels.

Although the initiation of CPB significantly decreased total diltiazem concentration in plasma (~50%), the level of unbound (free) diltiazem and the deacetylated and *N*-demethylated metabolites were not significantly changed. The plasma unbound fraction (FF) value increased sharply from 0.43 ± 0.12, prior to the onset of CPB to a peak value of 0.83 ± 0.12 during CPB and returned to baseline level 24 hours after dosing. The reduced plasma protein binding of diltiazem resulted in an increased FF and thus, nullified the effect of CPB on unbound drug concentration. The change in plasma protein binding could be attributed mainly to hemodilution and adsorption of α_1-acid glycoprotein to the CPB equipment, such as an oxygenator. Thus, the levels of unbound diltiazem during and immediately after CPB were equivalent to that normally present in the patients receiving diltiazem chronically. It is concluded that supplemental diltiazem is not needed during surgery. It is equally clear that if unbound drug levels are to be maintained after surgery then supplemental diltiazem should be given after CPB. Results in this study also indicate that measurements of both bound and unbound drug concentration is important to determine the effect of CPB on drug disposition.

The mechanisms of time-dependent pharmacokinetics of diltiazem was investigated using a single-pass isolated perfused rat liver model. Diltiazem, a tertiary amine, like

other tertiary amines such as lidocaine, verapamil and orphenadrine (a diphenhydramine analog) shows time-dependant pharmacokinetics in the human (*Smith et al. 1983, Bauer et al. 1982, Schwartz et al. 1985, Labout et al. 1982*). Diphenhydramine analogs are capable of inactivating some isozymes of P450 (*Bast et al. 1990*). This inactivation of enzymes has been postulated as the reason for their decreased clearance during chronic dosing, the time-dependent pharmacokinetics. Studies have shown that lidocaine is also capable of inactivating P450 isozymes involved in the *N*-dealkylation of lidocaine (*Saville et al. 1989*). Given the similarities in chemical and pharmacokinetic properties, diltiazem was postulated to have similar effects on the *N*-dealkylating isozymes.

We found that the binding capacities of diltiazem and its metabolites are high; this phenomenon is mainly responsible for the time-dependent kinetics of diltiazem in rats, inactivation of P450 isozymes involved in the N-dealkylation plays only a minor role (chapter 4). Hepatic tissue binding of diltiazem can explain, at least partially, the time-dependent kinetics in the human. Tissue binding is not unique to diltiazem but happens to other lipophilic tertiary amines such as lidocaine (*Saville et al. 1992*), imipramine (*Earlandsen and Gram 1982*)) and diphenhydramine (chapter 4, unpublished results). The binding capacity of diltiazem and/or its metabolites is 669.5 $\pm$ 156.5 and 974.2 $\pm$ 99.2 nmol/g of liver at C_{in} of 35.5 $\pm$ 3.2 and 67.2 $\pm$ 3.4 μM of diltiazem, respectively (chapter 4), while the binding capacity of lidocaine is 163.0 $\pm$ 95.0 nmol/g liver at C_{in} of 36.9 $\pm$ 4.6 μM (*Saville et al. 1989*). These values show that diltiazem binds more to the liver tissue than does lidocaine. This is also reflected in the partition co-efficient of diltiazem and lidocaine in the liver tissue versus buffer solution in the sinusoid (8.9

± 0.8 versus 5.0 ± 1.3). This indicates that at steady state, the liver tissue contains approximately nine and five times higher concentrations of diltiazem and lidocaine, respectively, than does the corresponding vascular concentration. Since diltiazem and lidocaine have an identical pK_a value (7.7) and an octanol/water partition co-efficient ($\log_{10} P = 2.3$), the above observations suggest that diltiazem has more affinity for the binding sites in the liver than lidocaine does.

The characteristic maximum in the concentration time profile of *N*-demethyldiltiazem (MA) and some irreversible binding of diltiazem to microsomes were attributed to the inactivation of enzymes involved in the *N*-dealkylation of diltiazem. In contrast to rats, the *N*-dealkylation is a major pathway for the metabolism of diltiazem in the human. Therefore, the contribution of enzyme inactivation to the time-dependent kinetics of diltiazem can be important in the human.

In our study, rates of deacetylation and *N*-demethylation, the former being the major pathway, were linear. This suggests that saturation of metabolism was not important for the observed time-dependent pharmacokinetics of diltiazem. Chronic diltiazem treatment does not alter liver blood flow in the human (*Bauer et al. 1986*). In our study, the perfusion rate was kept constant for all the livers. Thus, changes in liver blood flow cannot be a significant determinant of the time-dependent kinetics of diltiazem.

The effect of other tertiary amines on the kinetics of diltiazem was investigated using the single-pass isolated perfused rat liver model. Since there is a good possibility of administering diltiazem with lidocaine or diphenhydramine to patients with cardiac

diseases, we proceeded to evaluate the effect of lidocaine or diphenhydramine on the kinetics of diltiazem. We found that both lidocaine and diphenhydramine are capable of interacting with diltiazem when each is administered with diltiazem. These interactions are a combination of displacement of diltiazem from its tissue binding sites in the liver and inhibition of diltiazem metabolism. These results suggest that the three compounds share common isozymes in their disposition. In addition, diltiazem and lidocaine are capable of inactivating similar isozymes. We also found that diltiazem inhibits the metabolism of lidocaine and diphenhydramine during coadministration. Concurrent administration of diltiazem and lidocaine in the dog produced depressed cardiac function (*Kapur et al. 1988*). The authors could not conclude whether the observation was a result of pharmacokinetic and/or pharmacodynamic interaction. Our observations in isolated perfused rat liver clearly show that diltiazem and lidocaine interacts pharmacokinetically when given together. This interaction can have potentially serious and toxic side effects. This type of drug interaction between tertiary amines can be clinically important and deserves further study.

The effect of enzyme inactivation on the kinetics of diltiazem was studied by pretreating rats with diltiazem, lidocaine, diphenhydramine and verapamil. The pretreatment with these tertiary amines did not affect the kinetics of diltiazem. However, pretreatment with diltiazem and lidocaine resulted in a higher steady state material balance. This increase was associated with increased steady state levels of deacetyl, *N*-demethyl and *O*-demethyl metabolites of diltiazem. Based on these data, it can be speculated that further metabolism of these metabolites by unknown metabolic pathways

are inhibited. Since *N*-demethylation is a minor metabolic pathway for diltiazem in rat, pretreatment with tertiary amines does not significantly affect the kinetics of diltiazem although evidence of inactivation of *N*-demethylation was shown.

The mechanisms of time-dependent pharmacokinetics and drug interactions of diltiazem with other tertiary amine drugs have been thoroughly investigated in this project. Important observations on tissue binding and enzyme inhibition have been made that may explain many interactions between diltiazem and other drugs. This information is valuable for future studies involving time-dependent pharmacokinetics and drug interactions between tertiary amines.

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