UNIVERSITY OF ALBERTA

THE EFFECTS OF HYPERLIPIDEMIA ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF NIFEDIPINE

BY

LISE A. ELIOT

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

PHARMACEUTICAL SCIENCES (PHARMACOKINETICS)

Faculty of Pharmacy and Pharmaceutical Sciences

Edmonton, Alberta, Canada

Spring, 1999

"Nothing in the world can take the place of persistance -

talent will not, genius will not, education will not.

Persistance and determination alone are omnipotent."

- Calvin Coolidge

Dedication

This thesis is dedicated to my mom, since her belief in me has given me the motivation to start, continue and finish this thesis, to my dad whose famous words "don't sweat the small stuff (and it's all small stuff)" kept me from letting the small stuff get to me, to my brother for his constant laughter, and to my husband Keith whose wisdom and serenity has led me to the lighter side of life.

Abstract

Hyperlipidemia has altered the pharmacokinetics and pharmacodynamics of many drugs. Previously, decreasing total plasma cholesterol in hyperlipidemic subjects by using HMG CoA reductase inhibition has normalized the pharmacokinetic parameters. However only one study has evaluated these effects.

Naive (normolipidemic) and poloxamer-induced hyperlipidemic rats were dosed with nifedipine by iv, ip and po routes to study the effects of hyperlipidemia on the gastrointestinal and hepatic elimination of nifedipine. In addition, the fraction unbound of nifedipine in plasma was determined in both hyperlipidemic and normolipidemic rat plasma. Hyperlipidemia decreased hepatic clearance of nifedipine (39%) without altering gastrointestinal metabolism, which correlates well with a 31% decrease in the fraction unbound of nifedipine in plasma.

P407-induced hyperlipidemia was inhibited using HMG Co A reductase inhibitors, atorvastatin and lovastatin, thereby decreasing total plasma cholesterol by approximately 57%. The inhibition was dependent on the dosing schedule, but not on the HMG CoA reductase inhibitor.

Pharmacokinetic and pharmacodynamic evaluation comparing normolipidemic, hyperlipidemic, atorvastatin treated hyperlipidemic and atorvastatin treated normolipidemic rats corroborated with the previous PK results, demonstrating that hepatic elimination in hyperlipidemic rats was decreased. In addition, hyperlipidemia caused a sustained reduction of mean arterial due to an iincrease in total plasma nifedipine concentrations, despite a decrease in unbound nifedipine

concentration. Atorvastatin intervention decreased total plasma cholesterol normalizing the hepatic clearance of nifedipine. As well, the hyperlipidemic-induced lowering of mean arterial pressure was reduced although not completely.

A high incidence of hyperlipidemia exists in patients receiving nifedipine since it is commonly used in subjects with coronary heart disease. Although these studies only evaluated the calicum channel blocker nifedipine, and the rat model was the animal model chosen, these observations might have relevance in human studies with many calcium channel blockers that bind to lipoproteins. Possibly, total plasma cholesterol levels should be monitored to assess any potential alterations in nifedipine pharmacokinetics and the pharmacological response(s).

Acknowledgements

Thank-you Dr. F. Jamali, my mentor and supervisor, for accepting me into your laboratory and supporting and encouraging creativity. Most importantly, thank-you for your honesty and constructive critcisim, your words will be remembered and thought of always.

Special thanks to my very good friend and colleague Dr. John Grundy for the invaluable lessons on HPLC, surgical techniques, in vivo PK studies, and data analyses. Thank-you Dr. Pat Mayo for the ECG lessons, intriguing PK and PD conversations, and for always being there to talk to over a taco salad. I would also like to thank my fellow colleagues; Dr. Robert Carr, Dr. Keith Anderson, Sharon Mitchell, Ken Kulmatycki, Dr. Tahereh Khazaeinia, Micheal Giurgius, Dr. Jaber Emamibafrani, and previous lab manager Dr. Ray Lovlin for their participation in daily activities in the laboratory, helpful suggestions, and for the good times at the power plant.

Thank-you Greg Olson for being at my disposal for total plasma cholesterol analysis. In addition, thank-you Dr. Nick Nation at HSLAS for participating in the pathological assessments of the rats.

Last, but not least, I would like to express my sincere gratitude to my incredibly patient and supportive friends Dr. Stacey Roberts, Sue Lucenko, Theresa Baba, Cathy Lapinski, and Starr Grundy all of whom endlessly listened and supported each and every endeavor. Thank–you to my mother and father in-law, Janet and Edward Anderson for the wonderful hospitality not only for myself, but my family as well and for the unconditional support and understanding.

TABLE OF CONTENTS **Page No.**

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations / Nomenclature

~	Approximately
λ	Lambda – elimination rate constant
%Δ	Percent change
°C	Degrees Celcius
AAG	α_1-acid glycoprotein
ACAT	Acyl Coenzyme A:cholesterol acyltransferase
ANOVA	Analysis of variance
$AUC_{(0-\infty)}$	Area under the plasma concentration-time curve from 0 min to infinity
$AUC_{(0\text{-last})}$	Area under the plasma concentration-time curve from 0 min to the last measurable concentration
$AUC_{T(0\text{-}30)}$	Area under the plasma total concentration-time curve from 0 to 30 min
$AUC_{F(0\text{-}30)}$	Area under the plasma unbound concentration-time curve from 0 min
$AUEC_{(0\text{-}30)}$	Area under the effect-time curve from 0 min to 30 min
$AUMC_{(0-\infty)}$	Area under the first-moment plasma concentration-time curve from 0 min to infinity
B:P	Blood to plasma ratio
bpm	Beats per minute
C_{last}	Last concentration of a concentration-time profile
C_{max}	Maximum concentration

C_t	Total plasma concentration
C_u	Unbound plasma concentration
cc	Cubic centimetres
CETP	Cholesteryl ester transferase protein
CL_H	Hepatic clearance
CL_{sys}	Systemic clearance
CL_{TB}	Total body clearance
CLu_{int}	Intrinsic clearance of unbound drug clearance
$CL/F_{(ip)}$	Apparent intraperitoneal clearance
$CL/F_{(po)}$	Apparent oral clearance
CYP450	Cytochrome P450
DBP	Diastolic blood pressure
E_{max}	Maximum effect
EC_{50}	Concentration at 50% of maximum effect
ECG	Lead I electrocardiogram
ER_H	Hepatic extraction ratio
F	Absolute bioavailability
F_L	Fraction of drug escaping hepatic elimination
f_{av}	Fraction available for elimination
f_{up}	Fraction unbound in plasma
f_{ut}	Fraction unbound in tissue
FFA	Free fatty acids
Fig.	Figure

G	Gauge
h	Hour(s)
HDL	High density lipoprotein
HLE	Hepatic lipase
HLB	Hydrophilic/lipophilic balance
HMG CoA	3-hydroxy-3-methylglutaryl coenzyme A
HPLC	High Performance Liquid Chromatography
HR	Heart rate
IDL	Intermediate density lipoproteins
iv	intravenous
ip	intraperitoneal
kg	kilogram(s)
Ka	Affinity of plasma protein to drug, or association constant of plasma protein-drug complex
L	Litre
LCAT	Lecithin Coenzyme A:acyl transferase
LDL	Low density lipoproteins
LPL	Liprotein lipase
m	Slope of the concentration-effect profile using the linear effect model
M	Molar concentration
MAP	Mean arterial pressure
mg	Milligram

min	Minute(s)
ml	Millilitres
mmol	Millimoles
MTBE	Methyl-t-butylether
n	Hill's coefficient
n.d.	Not determined
N_{TOT}	Plasma protein capacity
nm	nanometres
p	Probability value or the degree of rarity of a test result, given that the null hypothesis is true
pH	Negative logarithmn (base 10) of the hydrogen ion concentration
P407	Poloxamer 407, Pluronic F127
PD	Pharmacodynamic
PEG	Polyethylene glycol
pharmacological $t_{½}$	Pharmacological half-life
PLTP	Phospholipid transfer protein
PK	Pharmacokinetic
SBP	Systolic blood pressure
Q_H	Hepatic blood flow
r	Correlation coefficient
r^2	Correlation coefficient squared

SD	Standard deviation
SEM	Standard error of the mean
t_{last}	Time of last concentration
t_{max}	Time to maximum concentration
$t_{½}$	Half-life
TCH	Total plasma cholesterol
TEA	Triethylamine
TG	Triglyceride
µg	microgram
U1	Total concentration in ultrafiltrate after initial filtration
UV	Ultraviolet
V_p	Volume of plasma
V_t	Volume of tissue
Vd_{ss}	Volume of distribution at steady state
VLDL	Very low density lipoproteins
v/v	Volume per volume
wk	Week(s)
w/w	Weight per weight
W1	Total concentration in ultrafiltrate after first buffer wash
W2	Total concentration in ultrafiltrate after second buffer wash
W3	Total concentration in ultrafiltrate after third buffer wash
W4	Total concentration in ultrafiltrate after fourth buffer wash
W5	Total concentration in ultrafiltrate after fifth buffer wash

Chapter 1

Introduction

1.1 Cholesterol

Cholesterol is an essential component of mammalian cells, regulating and providing, in part, a source of energy for normal cell function (1,2). Of primary importance, cholesterol is the metabolic precursor for *de novo* synthesis of steroid hormones, bile acids and hepatic derived lipoproteins (VLDL, HDL) (3,4).

1.1.1 Synthesis

All replicating cells have the ability to synthesize cholesterol, however the majority of endogenously produced cholesterol is derived from the liver (1). The de novo synthesis of cholesterol occurs on the endoplasmic reticulum (2) by a series of more than 30 enzyme-catalyzed reactions (1), and is illustrated (5) in Fig. 1-1 (Refer to page 3).

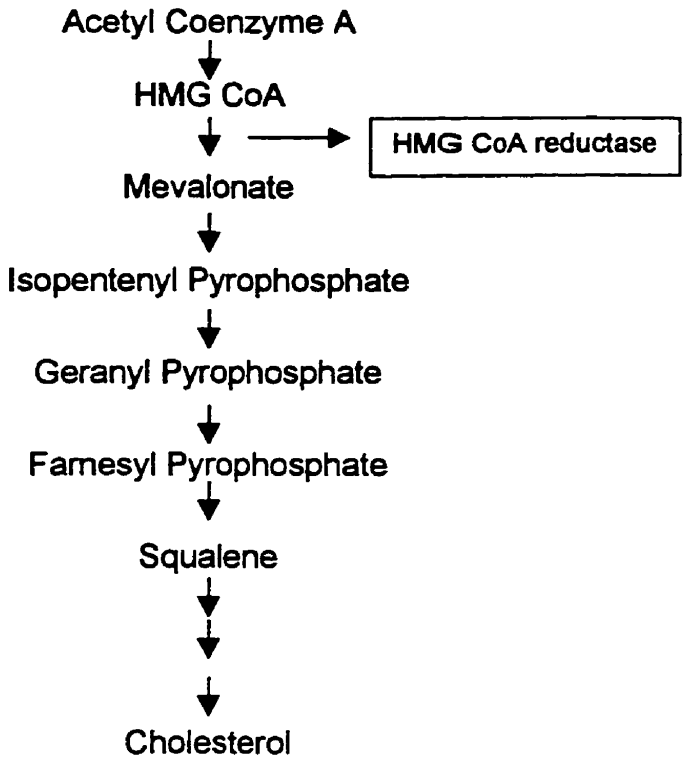

Fig. 1-1. *De novo* synthesis of cholesterol. Acetyl coenzyme A is condensed into 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA). The conversion of HMG CoA to mevalonate by HMG CoA reductase is the rate-limiting step in the *de novo* synthesis of cholesterol (1,6,7). Mevalonate is converted into isoprenes, which are precursors to units needed to form squalene. A microsomal mixed-function oxidase process converts squalene to lanosterol, proceeding a 19 step-reaction catalyzed by microsomal enzymes ultimately resulting in cholesterol (1).

1.2 Lipoproteins

Lipids and apolipoproteins are packaged into colloidal particles called lipoproteins, which enable transport of lipids in the bloodstream to surrounding tissues (8-10).

1.2.1 Structure and Composition

As Fig. 1-2 illustrates, lipoproteins are spherical particles composed of apolipoproteins, free (unesterified) and esterifed cholesterol, triglycerides and phospholipids. Lipoproteins are composed of two sections including the surface monolayer containing mostly polar lipids (free cholesterol, phopholipids, and apolipoproteins) and the internal core containing nonpolar lipids (esterified cholesterol and triglycerides) (1,11). Recent experiments have shown that free and unesterifed cholesterol and triglycerides may distribute between the external shell and internal core depending on the size of the lipoprotein (1,11). Nonetheless, the majority of lipid remains firmly trapped in the core (10). The lipoproteins may differ in density, size and lipid composition, depending on their physiological role(s) (1,11). The major categories of lipoproteins include chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

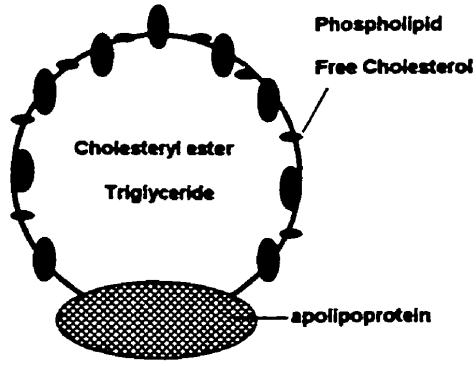

Fig. 1-2. Demonstration of the distribution of lipid and protein on the surface monolayer and within the internal core of a lipoprotein.

1.2.1.1 Human versus rat

As shown in Table 1-1 (See page 6) lipid and apoliprotein composition is distributed among the lipoprotein sub-classes substantially differently in the rat in comparison to the human.

Table 1-1. Comparisons of lipid and apolipoprotein composition among the different lipoprotein sub-classes (VLDL, LDL and HDL) between the human and rat species.

Properties	Species	VLDL	LDL	HDL
Density (g ml^{-1})		< 1.006	1.019 - 1.063	1.063 - 1.21
% Plasma TCH	Human (12,13)	10 -15	60 - 70	20 - 30
	Rat (14,15)	20 -30	10 -15	60 - 70
% Lipid Composition	Human (16)			
	TCH	12 -22	45	30
	TG	50	9	8
	Rat (17,18)			
	TCH	5 - 7	34 - 37	34
	TG	63	20	6
Apolipoprotein Composition	Human	B100, E, C1,C2, C3	B-100	A1, A2,C1, C2,C3, E
	Rat	B100, E, C	B-100	Al, A4, C, E

Total plasma cholesterol (TCH) and triglycerides (TG).

1.3 Lipoprotein Synthesis, Transport and Metabolism

1.3.1 Exogenous Pathway

Exogenous cholesterol and triglycerides enter the body through the diet, which are subsequently packaged into chylomicrons in the endoplasmic reticulum of enterocytes, entering the systemic circulation by an exocytotic mechanism via the lymphatics. Once in the systemic circulation, chylomicrons interact with HDL initially acquiring and finally losing apolipoproteins C and E.

The transfer of apolipoprotein C on the surface monolayer of HDL is necessary for the attraction and possible activation of lipoprotein lipase (LPL). On the vascular endothelium chylomicrons bind to LPL, producing a cholesterol rich lipoprotein "chylomicron remnant" after depletion of triglycerides. LPL is an enzyme that hydrolyzes triglycerides into free fatty acids. The chylomicron survives in the plasma for 2 - 5 min prior to catabolism by the liver. Receptor-mediated endocytosis following LDL receptor or chylomicron-receptor binding, at the liver, precedes catabolism of the chylomicron remnant. Binding of the chylomicron to the LDL receptor is mediated by apolipoprotein E (19). The chylomicron-remnant receptor is unaffected by cholesterol accumulation and causes cholesterol to accumulate to high levels in the liver when the diet contains excess fat.

1.3.2 Endogenous Pathway

1.3.2.1 Synthesis, uptake and catabolism of apolipoprotein B containing lipoproteins (VLDL, VLDL remnants, LDL)

VLDL is synthesized and assembled in the endoplasmic reticulum of the hepatocyte with both exogenous and endogenous derived cholesterol and triglycerides. As Fig. 1-3 illustrates (see page 12) the nascent VLDL is released into the bloodstream interacting with LPL and HDL producing the "VLDL remnant". IDL is considered to be a small VLDL remnant. In the human approximately 50 percent of the VLDL remnants are further processed into LDL. Although this process has not been unequivocally established, it is thought that continual lipolysis by LPL and hepatic lipase (HLE) completely depletes

triglyceride and apolipoprotein C and E from VLDL remnants, producing the cholesterol-rich apolipoprotein B-100 lipoprotein, LDL. The plasma VLDL remnants and LDL return to the liver for catabolism (8). The major uptake pathway of apolipoprotein B100 lipoproteins (VLDL remnants, LDL) is through a receptor-mediated endocytotic process governed by LDL receptors (10,20), which are found on the ovary, kidney, adrenal gland, liver and peripheral cells (fibroblasts and smooth muscle cells), but most are present on the liver (10). The LDL receptor binds apolipoprotein B-100 and E containing lipoproteins (VLDL remnants, LDL) forming an endosome, and very quickly (3 – 5 min) the lipoprotein is released from the endosome and delivered to a lysosome where apolipoproteins and cholesteryl esters are hydrolyzed to amino acids and cholesterol, respectively (10). The LDL receptor goes back to the cell surface to complete several hundred round-trips, within its 20 h life span. The residence time of VLDL and LDL is 15 – 60 min and 24 – 75 h, respectively (11). Once LDL receptors are saturated (10), the removal rate of LDL is proportional to the number of receptors. Whenever the number of receptors is reduced, plasma LDL levels will rise. In many species, including human, the consumption of a high fat diet decreases number of LDL receptors in the liver (10), resulting in hyperlipidemia.

Although VLDL contains apolipoprotein B100 and E, the presence of apolipoprotein C sterically inhibits the binding domain rendering it incapable of interacting with the LDL receptor (11). However, in 1992 a receptor which had greater affinity for VLDL than LDL was identified (21). Although VLDL tissue

uptake has not been conclusively shown to involve the VLDL receptor, VLDL tissue distribution is highly suggestive of triglyceride transport into metabolically active tissues. The heart, skeletal muscle and liver lack VLDL receptors. In addition to the specific receptor-mediated lipoprotein uptake pathway for the apolipoprotein B lipoproteins, two other minor pathways take place (8): (i.) Non-specific fluid-phase endocytosis (pinocytosis) by vascular cells and (ii.) Non-specific adsorptive endocytosis.

1.3.2.1.1 Human versus rat

In both rats and humans remove lipoproteins are removed from plasma similarly via receptor-mediated uptake (LDL receptors) (18). However, plasma concentrations of LDL are lower in normolipidemic rats since hydrolysis of VLDL remnants to LDL occurs less frequently in rats than in humans, possibly due to a greater efficiency of elimination of VLDL remnants in the rat (1,17). (Fig. 1-3, see page 12).

1.3.3 HDL – Reverse Cholesterol Transport and Lipid Transfer

Nascent HDL is secreted as a lipid-poor apolipoprotein A particle by the liver and gastrointestinal tract, and which acquires cholesterol from chylomicrons and peripheral cells, and apolipoprotein C from chylomicrons. Lecithin:cholesterol acyltransferase (LCAT) esterifies cholesterol on the surface monolayer of HDL, generating a chemical potential gradient that leads to continual uptake of circulating cholesterol. The cholesterol rich HDL exchanges esterified cholesterol and phospholipids for triglycerides with VLDL and LDL through a protein transport process mediated by cholesterol ester transfer protein (CETP) and

phospholipid transfer protein (PLTP) (8,22). (Fig. 1-3, see page 12). In addition, the cholesterol rich HDL delivers cholesterol to the liver for elimination by a process called "reverse cholesterol transport" (23,24). The process of uptake of HDL within the liver is rather different than that of the apolipoprotein B containing lipoproteins (VLDL remnants, LDL), whereby the majority of HDL uptake is receptor independent (11). Possibly a minor quantity of HDL are taken up into the liver via a receptor-mediated endocytosis, which appears to be dependent on the presence of apolipoprotein E.

1.3.3.1 Human versus Rat

The gradient that promotes reverse cholesterol transport is created by cholesterol transfer from HDL to LDL and VLDL remnants by CETP in the human (17,25). In rat however, the gradient is produced by liver uptake of the cholesterol rich HDL, since the rat lacks CETP activity (26-28). (Fig. 1-3, see page 12).

1.4 Cholesterol homeostasis

The major organ involved in cholesterol synthesis and elimination in the body is the liver. Furthermore, the liver is responsible for controlling the levels of cholesterol in the body. Since the body is subjected to continual input and output of large amounts of cholesterol (18,27) a rigorous homeostatic process is needed to maintain normal cholesterol levels. These homeostatic processes are accomplished through a number of carefully orchestrated mechanisms as follows (1,29):

- Decreased HMG CoA reductase expression,
- Increased acyl CoA: cholesterol acyltransferase (ACAT) activity,

- Down-regulation of LDL receptors.

1.4.1 Human versus Rat

The differential regulation of the aforementioned homeostatic mechanisms and their co-dependent interrelationships may account for the high degree of inter- and intraspecies differences in total plasma cholesterol concentrations in the blood.

The rat uses different mechanisms to maintain a normal basal total cholesterol level in the liver than human. In doing so, the rat is much less susceptible to elevations in total plasma cholesterol, which may explain the low total plasma cholesterol levels (0.5 - 2.5 mmol L^{-1}) (29,30) in the normolipidemic rat in comparison to the normolipidemic human (2.6 - 5.2 mmol L^{-1}) (24,31,32). For example, in response to a high fat diet, the human liver produces a decrease in hepatic synthesis by down-regulating HMG CoA reductase and decreasing uptake of cholesterol from plasma by down-regulating LDL receptors. As Fig. 1-2 indicates (see page 12), down-regulation of the LDL receptors is not commonly observed after high fat diets in the rat (29,33), since the maintenance of liver cholesterol can be maintained by regulation of HMG CoA reductase alone (30). Only when the capacity of the HMG CoA reductase is exceeded is the regulation of LDL receptors decreased (18), as seen in a hyperlipidemic rat model (34). In both rat and human,

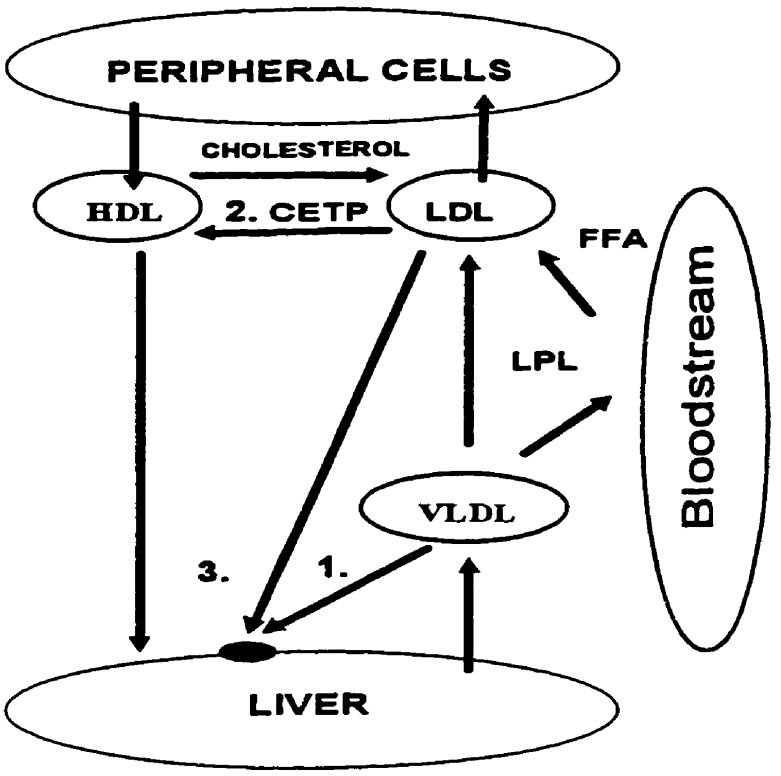

Fig. 1-3. Illustration of cholesterol (CH), triglyceride (TG) and lipoprotein transport, distribution and metabolism. TG is hydrolyzed to free fatty acids (FFA) via LPL. CH is transferred from VLDL remnants to HDL and from HDL to chylomicrons. TG is transferred from HDL to VLDL remnants and from chylomicrons from HDL. Differences between rat and human are designated as 1-3. (1). In the rat a greater amount of VLDL remnants are eliminated via the liver rather than being converted to LDL as it is in the human. (2). Rats lack CETP activity (3). LDL receptors in the rat are not as susceptible to increases in hepatic cholesterol as human, therefore down-regulation of LDL receptors are not as frequent as in human. Denotation a indicates an additional liver secretion and b indicates that the exact mechanism of uptake is not unequivocally established.

alterations in LDL receptor levels are not only under the influence of cholesterol, but also under humoral control (34,35), particularly the growth hormone. When the level of growth hormone is increased or decreased (hypophysectomy), LDL receptors are also increased (35) or decreased (36), respectively. The decreased frequency of LDL receptor down-regulation in rat may be due, in part, to higher amounts of growth hormone secreted in rat in comparison to human (36,37).

1.5 Hyperlipidemia

Hyperlipidemia is defined as an abnormal elevation in blood cholesterol, cholesteryl esters, triglycerides or phospholipids (24). Hyperlipidemia may be the result of a genetic defect (primary) or secondary to diet, disease, and drugs (32). The prevalence and large intra- and intervariability in total plasma cholesterol in the western civilization is most commonly due to the combination of one or more secondary causes with some degree of a primary defect (24). High fat diets, weight gain, smoking, alcohol, and lack of exercise are behavioral factors that are secondary causes of hyperlipidemia (24). Chronic diseases most commonly associated with hyperlipidemia are diabetes, hypothyroidism, growth hormone deficiency, anorexia nervosa, systemic lupus, and chronic renal failure (34,35). Acute conditions resulting in hyperlipidemia are nephrotic syndrome, pregnancy, inflammation, and bacterial and viral infections (24,38). Hyperlipidemia resulting from infection may be due a suppression of HLE and LPL produced by interferons, interleukin-1 and tumor necrosis factor (39,40). Also, total plasma cholesterol may be elevated after traumatic injury such as myocardial infarction, surgery, burns, and organ transplantation since lipoproteins are acute phase

reactant proteins (41,42). Drugs may also cause hyperlipidemia such as cyclosporine A, thiazide diruetics, nonselective β-blockers, and steroids.

1.5.1 Hyperlipidemia and Coronary Heart Disease

Atherosclerosis is a multifactorial disease occuring through a complexity of processes that are still under investigation. Regardless, it is widely accepted that risk factors include increases in total and LDL cholesterol and decreases in HDL cholesterol (43-45). On the contrary, elevated triglycerides have not conclusively proven to be an individual risk factor (44,46). However, in some disease states it has been associated with increased cardiovascular risks (32). It has been conceded that atherosclerotic lesions are derived from uptake of oxidized LDL by macrophages, leading to the earliest form of atheroma called the "foam cell" (32). The foam cell is the beginning of a multitude of processes that accumulate to form an atherosclerotic plaque.

1.5.2 Therapeutic Intervention

The benefits of lowering cholesterol in established coronary heart disease have been unequivocally proven (47,48). Large-scale studies using drug therapy to decrease total plasma cholesterol have demonstrated a reduction in coronary events (49,50), total and coronary mortality (48), progression of disease (49), and regression of atheroma (51). Although a single class of drugs could not possibly treat the numerous types of hyperlipidemia, HMG Co A reductase inhibitors are most extensively used (47).

1.5.2.1 HMG CoA reductase inhibitors

HMG CoA reductase inhibitors, such as lovastatin, simvastatin, pravastatin, fluvastatin and atorvastatin are commonly used to treat hyperlipidemic conditions of primary (heterozygous familial hypercholesterolemia, familial combined hyperlipidemia) and secondary origin (non-familial forms of hypercholesterolemia, combined hyperlipidemia, including familial combined hyperlipidemia and patients with non-insulin dependent diabetes mellitus) (47,52). The liver is the target organ for inhibition of HMG CoA reductase. Reduced synthesis and concentrations of hepatic cholesterol cause an upregulation of LDL receptors, thereby increasing plasma elimination of apolipoprotein B lipoproteins (VLDL, VLDL remnants and LDL) (53). The decrease in hepatic cholesterol also leads to a decrease in production of VLDL (54). The contribution of these mechanisms to the overall decrease in total plasma cholesterol is undetermined (55). Nevertheless, decreases in total plasma cholesterol, LDL-cholesterol, and -triglycerides, and increases in HDL-cholesterol are observed. Pharmacokinetic (PK) and pharmacodynamic (PD) relationships of the HMG CoA reductase inhibitors have not been established because the clinical effect, ie. lowering of cholesterol and triglycerides, is not instantaneous (56). However, dose has been positively correlated to cholesterol lowering activity (55,56). The HMG CoA reductase inhibitors have similar clinical effects, with the exception of atorvastatin. Atorvastatin, the most recently marketed inhibitor, is the most potent HMG Co A reductase inhibitor (57,58).

Atorvastatin, has also been shown to be a more potent cholesterol and triglyceride lowering agent than the other inhibitors. (58,59).

1.5.2.1.1 Atorvastatin and lovastatin

Lovastatin is a prodrug, being an inactive lactone that is converted by carboxyesterases in the liver and plasma to an active hydroxyacid (56). Atorvastatin is a calcium salt that exists in the active hydroxyacid form (55,60). Atorvastatin is sparingly soluble in aqueous media (61), whereas lovastatin is very water insoluble. Atorvastatin is readily absorbed (62), however due to pre-systemic elimination it has an oral bioavailability of only 12% (59,63). Similarly, lovastatin has a low oral bioavailability, due in combination to poor absorption and high hepatic extraction (56). Atorvastatin and lovastatin are highly protein bound, 98 and 95% respectively, and are each metabolized by cytochrome P450 (CYP450) 3A (56), particularly CYP450 3A4 (55,59). The half-life (t½) of elimination for atorvastatin and lovastatin in human is 13 - 51.5 h (61,63) and 1.5 - 2.9 h (64,65), respectively, but the t½ of inhibition does not reflect the drug $t_{1/2}$, perhaps due to active metabolites, or other factors.

1.5.3 Hyperlipidemic Rat Models

The eagerness to understand the pathogenesis and treatment of hyperlipidemia has led to the development of numerous hyperlipidemic models. There are many considerations when choosing a hyperlipidemic model, such as the ability to replicate the human condition, convenience, and expense. Despite the differences in lipoprotein metabolism, distribution and total plasma cholesterol levels between the human and rat, the rat is commonly used in lipid

research (66). Hyperlipidemic rat models vary in their etiology, and their type and severity of hyperlipidemia. The majority of the models reflect genetic alterations(s), including the JCR:LA corpulent (67), obese Zucker (68,69), ExHC (70), RICO (71), Spontaneously Hyperlipidemic (66), and Prague Hereditary Hypercholesterolemic rats (14), many of which are expensive and have underlying pathological conditions. Feeding high cholesterol diets to rats does not commonly result in high plasma cholesterol levels, with the result that this model is not commonly used. Hyperlipidemia induced by the surfactant, Triton WR 1339 (72), has been recognized since 1951 and is currently being used to determine rates of VLDL secretion from the liver in order to characterize other hyperlipidemic models (73). Triton WR 1339 causes cell lysis, which limits its use (74).

1.5.3.1 Poloxamer 407

1.5.3.1.1 Physicochemical properties

Poloxamer 407 (P407, Pluronic F127) is a member of the poloxamer family, composed of repeating polyoxyethylene and polyoxypropylene blocks (75). P407 is a non-ionic surfactant and biocompatible polymer, and its molecular weight and HLB (hydrophilic-lipophilic balance) are 12,600 and 18 - 23, respectively (76). P407 is a high molecular weight poloxamer and contains a large content of ethylene oxide (hydophile) in comparison to other poloxamers, which contributes to its non-toxic nature (76). P407 undergoes a reverse thermal gelation, changing from a viscous liquid to a semisolid gel, which is dependent on P407 concentration

and temperature. For example, the semisolid gel liquifies at 30% w/w in water at 11°C (77).

1.5.3.1.2 Uses

P407 is used as an excipient in parenteral formulations. It is employed as a solubilizing and wetting agent (75), and a stabilizing agent for proteins and polypeptide drugs (78,79). The advantages of detergents in protein delivery include prevention of protein adsorption to surfaces, inhibition of protein aggregation and precipitation, and reduction in protein denaturation (78). Nonionic detergents have also been reported to facilitate the reactivation of denatured enzymes (78).

1.5.3.1.3 P407-induced hyperlipidemia in rat

P407 induces a dose-dependent, reversible elevation of total plasma cholesterol and triglycerides. One g kg^{-1} ip dose produces a 8 - 11 and 30 – 50 fold increase in total plasma cholesterol and triglyceride concentrations, respectively, 48 h after administration, which declines to baseline levels between 96 - 120 h (15,80). The mechanisms involved in P407-induced hyperlipidemia are stimulation of HMG CoA reductase (15,81,82), inhibition of LPL (80), and increases in mobilizaton of free fatty acids from adipocytes (83). The exact contributions of the individual mechanisms to the overall increase in total plasma cholesterol and triglyceride have not been established.

1.5.3.1.4 Disposition of P407

Until recently, attempts have not been made to characterize P407 disposition due to the absence of a suitable assay. In 1996, a colorimetric assay was

developed for these purposes and the plasma-concentration time profile of a 1 g kg^{-1} dose was described up to 48 h (84). The maximum concentration (C_{max}) of 13.5 mg ml^{-1} occurred at 12 h, that slowly declined to 4.5 mg ml^{-1} at 48 (84,85). It has been assumed that renal excretion of P407 is the main elimination pathway, because 75% of an intra-arterial dose given to dogs was recovered intact in the urine (84). This, however, has not been confirmed for the rat (84). In addition, at 24 h, liver and kidney homogenates contained 15.9 and 3.0 mg of P407, respectively. The apparent clearance of P407 was low (2.4×10^{-3} L hr^{-1} kg^{-1}), which was suggested to be due to deep tissue accumulation, protein binding or reabsorption in the distal tubules (84). From urinary data, the t½ of P407 has been reported to be 20.9 h (84), which correlates well with the hyperlipidemic effects.

1.5.3.1.5 Toxicity of P407

Surfactants are commonly known for their ability to disrupt membrane integrity, possibly causing cell lysis and altering cellular function (86). It has previously been shown that P407 is non-toxic to cell membranes (79,87,88). Toxicological studies have been conducted to determine the physiological effects of high P407 doses. In the rat, 4 days of once-daily administration of 0.33 mg kg^{-1}, ip, resulted in an increase in the number of monocytes with no other toxicological findings (89). In contrast, a dose of 1 g kg^{-1}, ip, given under similar conditions, decreased weight gain, altered blood cell counts, and produced a slight splenomegaly (89). Decreases in lymphocytes, erythrocytes, hemoglobin, and hematocrit, and increases in white blood cells and monocytes were noted.

Splenomegaly was caused by an infiltration of macrophages containing phagocytized lipids.

1.6 Protein binding

Human plasma contains over 60 proteins, the most abundant of which is albumin (90). The majority of drug binding is by albumin, α_1-acid glycoprotein (AAG) and lipoproteins (91). Albumin predominantly binds acidic entities (92), whereas AAG and lipoproteins bind basic and neutral entities (93). The prevelance of plasma binding and selectivity of proteins varies widely for each drug and is dependent on the following: (i) physicochemical properties of the drug, (ii) concentration of protein, commonly referred to as the protein's capacity (N_{TOT}), (iii) strength or affinity of binding of protein to drug (Ka) and (iv) concentration of drug (81,94). Some drugs may bind specifically to one protein (warfarin, probucol) while others may bind to all three (propranolol, nifedipine).

1.7 Alterations of PK and PD Profiles in Hyperlipidemia

Many drugs, particularly hydrophobic entities, associate to plasma lipoproteins (95). In previous reports, a decrease in fraction unbound in plasma (f_{up}) has occurred for many drugs in a hyperlipidemic condition including: cyclosporine A (96) (97), imipramine (98), amitrityline (99), nortriptyline (99) and fentanyl (100). In addition, inverse correlations between f_{up} and total plasma cholesterol and/or triglyceride levels have been reported (96,101,102,102-104).

The decreases in f_{up} were caused either a decrease in total body clearance (Cl_{TB}) (103,105,106), a decrease in volume of distribution (Vd_{ss}) (97,103,106,107), or an increase in drug plasma concentrations (105-113). In

contrast, few studies have reported an increase in Cl_{TB} and Vd_{ss} (114,115). Drug intervention to lower total plasma cholesterol has corrected the hyperlipidemic alterations in the PK parameters (101)

Hyperlipidemia may produce a multitude of differing effects on a drug's pharmacological response(s). Studies investigating the clinical consequences of increased plasma drug concentrations in hyperlipidemia reported either a decrease in expected toxicities (109,110) or no alterations in clinical efficacy for the indicated use (108). Other studies have shown that increased associations of drugs with lipoproteins causes either an increase (116,117) or a decrease in toxicity (96,118-120), depending on the particular lipid or lipoprotein involved. These conflicting findings are most likely explained by: (i) differences in the structure and makeup of the lipoproteins that influence the selectivity of binding to a particular lipoprotein (22,121-123), (ii) multiple pathways of lipoprotein distribution, uptake, and metabolism (110,116,124-126) and (iii) exchange of drug with lipids and proteins between the lipoproteins (22).

1.6.1 Drug Lipoprotein Interactions.

There is substantial evidence to support two mechanisms of lipoprotein-drug interactions, including: (i) saturable association, which is specific to either the lipids or proteins on the surface monolayer (95,118,127), or (ii) unsaturable association, which involves drug solubilization within the internal core of the lipoprotein (95,97,102,128). The type and extent of drug binding is influenced by the physicochemical properties of the entity in question. Neutral and basic compounds undergo 50 – 100 percent and 5 – 60 percent lipoprotein binding,

respectively (95), which primarily depends on the lipophilicity of the compound (95). In addition, the extent and distribution of lipoprotein binding may depend on lipoprotein concentrations in plasma, lipid concentrations within the individual lipoproteins (22,95,102), and the particular apolipoprotein on the surface monolayer (96).

1.6.1.1 Calcium channel blockers – lipoprotein interactions

The calcium channel blockers that have been shown to bind to lipoproteins are diltiazem (41), nifedipine (129), nicardipine (102), isradipine (95), amlodipine (95), and darodipine (130), but they do not necessarily have similar determinants in lipoprotein binding. For example, isradipine binding correlates linearly with the cholesterol levels in VLDL and LDL, but not HDL. However, nicardipine binding (102) correlates with triglycerides and phospholipids within VLDL, LDL, and HDL (102). Nifedipine binds to both HDL and LDL (129), although the affinity of association of lipoprotein to drug (Ka) in hyperlipidemic plasma is not known.

1.6.4 Cytochrome P450 (CYP450)

The "fluid mosaic model" proposed in 1972 describes the fluid nature of amphipathic membrane bilayers that are composed of phospholipids, cholesterol and proteins. The fluidity of the membranes is imperative for maintaining proper integrity and functioning of cellular processes (131). Increases in cholesterol content increases the cholesterol-phospholipid ratio within the bilayers, and decreases the fluidity of the membranes (131-133). Free (unesterified) cholesterol is a major lipid class of mammalian plasma membranes, and is thought to participate in the regulation of the physical state or "fluidity" of the

phospholipid bilayer (134). Although the catalytic activity of the CYP450 system strongly depends on phospholipid concentrations (135), it seems that the phospholipid:cholesterol ratio is also important (134,136). Cholesterol modulates membrane structure due to its unique interactions with phospholipid molecules and is incorporated into the membrane hydrocarbon core where it reduces freedom of motion. The effect is attributed to the contribution of the electron rich conjugated ring structure of cholesterol, which disrupts the ordering of acyl chain regions within the bilayer, and causes increases in bilayer membrane density and bilayer thickness.

An increase in the cellular free cholesterol:phospholipid ratio has been shown to alter the activity of several membrane-bound enzymes in a variety of cells, including hepatic cells, erythrocytes, intestinal epithelial cells, and renal tubular cells (134,136). CYP450 isozymes are integral proteins spanning the microsomal membrane bilayer of the endoplasmic reticulum (137) and are the major metabolizing isozymes for xeniobiotics (138,139). The fluid environment that facilitates electron transport from NADPH via NADPH-CYP450 reductase to CYP450 may be reduced by increased cholesterol (135). In addition, hyperlipidemic conditions have down-regulated (137,140,141) CYP450 content and have decreased the enzymatic activities of components of the CYP450 dependent monoxygenase system (137,142,143).

1.7 Nifedipine

1.7.1. Uses

Nifedipine, a first-generation 1,4-dihydropyridine calcium channel blocker, has been used in clinical practice for many years. Nifedipine elicits its therapeutic effect through a potent vasodilator action by blocking the L-type calcium channels in smooth muscle (144-146). Nifedipine is indicated for use in hypertension and ischemic heart disease (147,148). Despite the ongoing debate concerning its benefits and safety, nifedipine (149-151) is one of the most commonly prescribed antihypertensive drugs (147,152). Over the last twenty years, nifedipine has been assessed for other indicated uses, such as control of unstable angina (153) and long-term prevention of acute myocardial infarction (154). Unfortunately, the conclusions of these studies are limiting because inclusion immediate release capsules were previously used, whereas, for the most part, nifedipine is now adminstered by sustained-release formulations (149). Sustained-release formulations (eg. Adalat PA or XL) have improved the PK and PD profiles of nifedipine by reducing C_{max} and increasing the time to reach C_{max} (t_{max}), which has generally produced a gradual and sustained lowering of blood pressure (155-157) and decreased the occurrence of reflex tachycardia, a baroreceptor feedback mechanism. To eliminate bias, large-scale clinical studies are continuing to determine the benefits of various nifedipine sustained release formulations (158). More recently, nifedipine was tested for potential anti-atherosclerotic properties due to the implication of calcium-dependent processes in plaque formation (159-161). Proposed mechanisms for the anti-atherosclerotic

properties are a reduction in smooth muscle proliferation, inhibition of growth factors, and other antioxidant or antiplatelet properties (159). Undoubtedly, nifedipine will be one of the most highly tested drugs in order to bring clarity to its clinical role (147).

1.7.2 Physicochemical Properties

The chemical structure of nifedipine ($C_{17}H_{18}N_2O_6$) is depicted in Fig. 1-4. The drug is practically insoluble in water with a saturated solubility of approximately 10 μg mL^{-1}, which is reduced to less than 6 μg mL^{-1} in phosphate buffer (162).

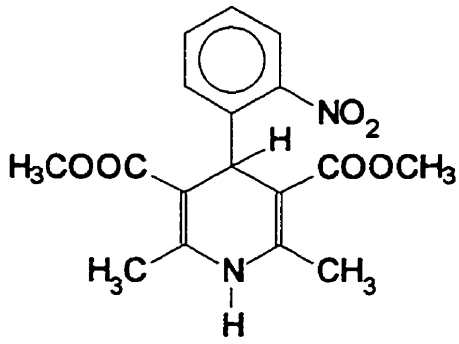

Fig. 1-4. Chemical structure of nifedipine.

The n-octanol:water partition coefficient is approximately 10 000:1 (162). Nifedipine is a weak base that is not charged at physiologic pH (163). The ultraviolet (UV) spectrum of nifedipine shows absorption maxima at 235 nm and about 340 nm in methanolic solution, and at 238 nm and about 340 nm in alkaline and acid solutions, respectively (162). Nifedipine is photosensitive, therefore it will degrade to inactive compounds upon exposure to visible and UV light (164). Therefore precautions must be taken when preparing nifedipine

solutions and samples by using yellow (sodium) or red light to prevent degradation.

1.7.3 PK and PD of nifedipine

Absolute bioavailability of nifedipine is 0.40 - 0.64 in both the rat and human (165-167). A substantial amount of pre-systemic elimination occurs in the gastrointestinal tract and liver (167-169). Nifedipine is metabolized to inactive metabolites by oxidative processes via CYP450 3A isozymes (168,170). In the rat, the systemic clearance (Cl_{sys}) is reported to be 2.4 - 10 ml min^{-1} kg^{-1}, indicative of a low to medium extracted drug, corroborated by a low to medium hepatic extraction ratio (ER_H) (0.22 - 0.47) (167,169). Albumin, AAG, and lipoproteins contribute to the high nifedipine plasma binding (91 - 99%) (129,171).

Diseases that alter nifedipine protein binding and/or metabolism generally alter the PK and PD profiles (172-174), evidenced by positive relationships between nifedipine concentration and both blood pressure lowering and toxicity (175). Concentration-effect relationships for blood pressure lowering of nifedipine have been defined by the linear effect and sigmoidal-maximum pharmacological effect (E_{max}) models (175). These relationships are formulation dependent, as demonstrated by comparisons of immediate- and sustained-release formulations (155,156).

1.8 Thesis Rationale

Distribution, elimination and pharmacological effects of many drugs have been altered by hyperlipidemia (22,47). Plasma lipoproteins, carriers of cholesterol and triglycerides, have the potential to interact with drugs, particularly

hydrophobic entities. They exist in plasma at 10% of the concentration of albumin (91). Regardless, they can account for the majority of total drug binding in some entities (97,176). In hyperlipidemic conditions, total plasma cholesterol and triglycerides are elevated, thereby increasing lipoprotein concentrations and their respective lipid masses therein. An increase in protein binding results in a decrease in f_{up}. Also, in the hyperlipidemic condition decreases in the content and activities of hepatic metabolizing isozymes, particularly CYP450, have been reported, which may cause a decrease in Clu_{int}. Both a decrease in f_{up} and Clu_{int} could alter the time-dependent C_t and C_u of nifedipine, which may alter its desired clinical effects. In addition, lipoproteins may re-distribute drugs away from effect sites to alternate organs (kidney, ovary), causing desirable pharmacological actions (adverse effects) and/or altering toxicities.

Many calcium channel blockers, including nifedipine, (129) associates with lipoproteins (41,95,102), however the PK and PD consequences have not been determined. Calcium channel blockers are used to lower blood pressure in patients with or without coronary heart disease. Hyperlipidemia is an independent risk factor for coronary heart disease. Therefore, patients being treated with calcium channel blockers may have an underlying hyperlipidemic condition. Nifedipine, a 1,4 dihydropyridine calcium channel blocker has been associated with adverse reactions when used in patients with established coronary heart disease, which may subsequently result in increases in cardiovascular events and mortality (151). Increased plasma concentrations of nifedipine correlates with nifedipine toxicity (156,177). Therefore, the presence of an underlying

hyperlipidemic condition may alter the PK and PD profiles of nifedipine in a way that may explain the large degree of interindividual variability (178) and increased risk in patients with established coronary heart disease.

1.9 Hypotheses

1. Short- and long-term administration of 1 g kg^{-1} P407 ip either once or for 4, 6 and 9 wk every 96 h is non-toxic.
2. Hyperlipidemia will alter the PK of a po, ip, and/or iv dose of nifedipine.
3. The f_{up} of nifedipine will be decreased in hyperlipidemic plasma.
4. HMG CoA reductase inhibitors will inhibit P407-induced hyperlipidemia.
5. Atorvastatin is a more potent HMG CoA reductase inhibitor than lovastatin in the P407-induced hyperlipidemic rat.
6. Pentobarbital sodium anesthetized rats will be a suitable model for determining the antihypertensive effects of nifedipine.
7. Intravenous (iv) nifedipine will demonstrate a sigmoidal dose-response relationship between the doses of 0.05, 0.1 and 0.3 mg kg^{-1} and blood pressure lowering.
8. Hyperlipidemia will either increase, decrease or not alter the blood pressure lowering effect of nifedipine.
9. Therapeutic intervention with atorvastatin in P407-induced hyperlipidemic rats will normalize PK and PD alterations caused by P407-induced hyperlipidemia.

1.10 Objectives

1. To evaluate the pathological and toxicological affects of short and long-term administration of P407 in the rat.

2. To evaluate the PK of an iv, ip and po dose of nifedipine in the P407-induced hyperlipidemic rat.
3. To compare the f_{up} of nifedipine in normolipidemic and hyperlipidemic rat plasma.
4. To evaluate and compare the effects of HMG CoA reductase inhibitors, atorvastatin and lovastatin, on P407-induced hyperlipidemia, using 3 different dosing schedules.
5. To evaluate the effects of pentobarbital-induced anesthesia on the mean arterial pressure (MAP) and heart rate (HR) in the rat to determine if this would be a suitable model to evaluate PD of nifedipine.
6. To evaluate the dose-response relationships of iv nifedipine using doses of 0.05, 0.1 and 0.3 mg kg^{-1}.
7. To evaluate the consequences of P407-induced hyperlipidemia on the PK and PD of nifedipine in the anesthetized rat.
8. To evaluate the consequences of cholesterol lowering in the hyperlipidemic rats, with atorvastatin, on the PK and PD of nifedipine in the anesthetized rat.

Chapter 2

Experimental

2.1 Chemicals

Nifedipine was obtained from Sigma Inc., (Mississauga, ON, Canada). Nisoldipine was a gift (Dr. Knaus, University of Alberta, Edmonton, AB, Canada). Methanol and water (HPLC grade), acetic acid (analytical grade) and triethylamine (TEA) were obtained from Mallinckrodt (Paris, KY, USA). Iso–octane and methyl–t–butyl ether (MTBE, HPLC grade) were purchased from BDH (Toronto, ON, Canada.). P407 was obtained from BASF Corporation Canada Inc. (Toronto, ON, Canada). Atorvastatin (Lipitor®, Parke-Davis, Scarborough, ON, Canada) and lovastatin (Mevacor®, Merck Frosst, QUE, Canada) were supplied as 40 mg tablets. Polyethylene glycol (PEG 400) was obtained from Union Carbide Chemicals (Danbury, CT, U.S.A.).

2.2 Dosage Form Preparation

Nifedipine solutions were prepared by dissolving nifedipine powder in PEG 400 the night prior to administration to ensure complete dissolution. To prevent nifedipine degradation, all dosage formulations were prepared and handled in a room illuminated only by sodium light. P407 was prepared as a 30% (w/w) solution in cold double distilled water 48 h prior to use and kept at 4-8 °C in order to maintain a liquid phase. Tablets containing 40 mg of atorvastatin and lovastatin were crushed and suspended in (0.4% w/v) methylcellulose to a final concentration of 30 mg ml^{-1}, immediately prior to use. Methylcellulose solution was made by dispersing the powder in 60 °C double distilled water (1/3 of the total volume). The remainder of water (2/3 of the total volume) was cooled to 4-8 °C and added to the hot solution.

2.3 Animal Treatment

Experiments were performed on male Sprague-Dawley rats weighing between 198 - 400 g obtained from Biosciences Animal Services (University of Alberta, Edmonton, AB, Canada). Rats were subjected to light from 8:00 a.m. to 7:00 p.m. and dark for the remainder of time. Rats were allowed access to drinking water and regular rat chow Purina Mills Inc. 5001 diet (PMI Feeds Inc., St. Louis, MO, U.S.A.). at all times. The lipid components that were contained in the diet are as follows; fat (4.5%, ether extract), fat (5.5%, acid hydrolysis), cholesterol (200 ppm), linolenic acid (1.16%), arachidonic acid (<0.01%), omega-3 fatty acids (0.26%), total saturated fatty acids (1.50%) and total monounsaturated fatty acids (1.58%) with a total gross energy and metabolizable energy (4.00 and 3.04 kcal/g, respectively). Rats were collected and kept in a study area 72 h prior to experiments where they had unlimited access to food and water *ad libitum*. In addition, food and water was given ad libitum to conscious rats throughout the entire duration of the experimental studies. All procedures followed the ethics of animal investigation.

2.4 Surgical Procedures

2.4.1 Anesthesia

All surgical procedures were carried out under surgical anesthesia, induced with 65 mg kg^{-1} of sodium pentobarbital (Somnotol®, MTC Pharmaceuticals, Cambridge, ON, Canada), and, if needed, maintained with 20 mg kg^{-1}. In unconscious rats maintenance doses of pentobarbital sodium were

administered directly into the ip cavity through a small midline incision in the lower abdomen.

2.4.2 Cannulations

Jugular vein cannulation was performed to provide a site for blood sampling and iv administration of nifedipine solution. In addition, carotid artery cannulation was performed to provide a site for blood pressure measurement. The jugular vein cannula consisted of 16 cm of polyethylene tubing with an internal and external diameter of 0.58 and 0.97 mm (PE-50, Clay Adams, Parsippany, NJ, U.S.A.), tipped with 2 cm of silastic tubing (Dow Corning Corp., Midland, MI, U.S.A.). The silastic tubing tip was inserted into the vein proceding the polyethylene tubing. Carotid artery cannulae were made with 9 cm PE-50 tubing cut to a bevel of about a ½ cm. The arterial cannula was inserted about 1 cm into the artery and secured into place with surgical sutures (Surgical Suture USP, Cyanamid Canada, Montreal, QUE, Canada.). To prevent release of heparin releasable LPL, an enzyme which is responsible for catabolism of triglycerides, the cannulae were locked with normal saline rather than heparinized saline.

2.4.3 Heart Rate and Mean Arterial Pressure Measurements

Lead I electrocardiograms (ECG) were produced by placing stainless steel teflon coated wiring (40G, Cooner Wire Co., Chatsworth, CA, U.S.A) subcutaneously, forming a triangle from either axilla to the xyphoid process. Heart rate (HR) was taken as the length of the R-R interval of the ECG. Carotid artery cannulae were connected to a Grass pressure transducer (Honeywell Phillips Org.,

U.S.A). Blood pressure and heart rate were displayed on a bridge amplifier (Honeywell, Electronics for Medicine, Edmonton, AB. Canada) and an IBM Personal Computer (IBM Inc., Armonk, New York, U.S.A). Acknowledge Version 3.0 (World Precision Instruments, Miami, FL, U.S.A.) was used to determine systolic and diastolic blood pressure from the pressure waves. Mean arterial pressure (MAP) was calculated by MAP = ((2 * DBP) + SBP) / 3 (179), where DBP is diastolic blood pressure and SBP is systolic blood pressure.

The pressure transducer was calibrated daily with a sphygmomanometer attached to 9 cm of polyethylene tubing that was directly attached to the pressure transducer. Calibration curves from 30 to 150 mm Hg were linear, $r^2 = 0.99$, and intra- and interday variability was less than 10%.

2.5 P407-induced Hyperlipidemic Rat Model

Hyperlipidemia was induced in rats by ip administration of 1 g kg^{-1} P407, 48 h prior to experimentation. Rats that served as controls received 1 ml saline in place of P407 in the identical manner. A small midline incision into the lower abdomen was made to provide access to the ip cavity for administration of P407. The incision was enclosed immediately using surgical sutures (Surgical Suture USP, Cyanamid Canada, Montreal, QC, Canada). Surgery was required because P407 could not be administered by a 26 G, 6.4 mm needle due to the thickness of the gel, therefore an 18 G, 19 mm needle was required which might traumatize surrounding organs. Needles and syringes were cooled prior to P407 injection to decrease gelation of P407 within the syringe.

2.6 Blood Sampling

Blood samples of approximately 150 - 250 μl were drawn through the jugular vein cannula with heparinized-coated 1 cc tuberculin syringes, unless stated otherwise. Immediately following blood sampling the cannula was rinsed thoroughly with blood, thereafter saline was administered to replace the lost blood. Note: the cannulas were not assayed for nifedipine after the intravenous injection, therefore initial concentrations found may be somewhat higher than the actual concentrations. However, the AUCs that were found in this thesis were comparable to previous findings (169). In addition, the tail clip method was utilized when only a few blood samples were needed (Please refer to sections 2.91 and 2.12.2). Approximately 2-3 mm of the tail was clipped using a sterilized razor blade. Immediately thereafter, silver nitrate sticks were used to stop the bleeding. Blood was centrifuged for 5 min at 2500 g to obtain plasma and samples were frozen at –20 °C until analyzed.

2.7 Analysis of Total Plasma Cholesterol

Total plasma cholesterol levels were determined by the Surgical Medical Research Institute, University of Alberta, Canada. The VETTEST 8008 (Innex Canada Corporation, Toronto, ON, Canada) dry chemistry analyzer was used which utilizes a colorimetric assay. The inter- and intra-day variability was less than 12%.

2.8 Analysis of plasma nifedipine

Plasma nifedipine concentrations using 50 - 150 μl of rat plasma were measured by a previously reported HPLC method (180). The internal standard

used was nisoldipine. The method involved extraction with MTBE:iso-octane (75:25, v/v) and subsequent evaporation of the resulting organic phase (about 90% of nifedipine is extracted from plasma within these concentrations). The residue was reconstituted with 200 μl mobile phase (methanol:water:acetic acid, 65:34:1, v/v plus an additional 300 μl of triethylamine). Approximately 150 μl of sample was injected onto a Nova-pak 8 x 100 mm radial pack column, containing 4μm C8 as stationary phase (Waters, Mississauga, ON., Canada). Nifedipine was detected by ultraviolet/visible at 350 nm using SPD-10A UV-vis detector (Shimadzu Corporation, Kyoto, Japan). Calibration curves were linear (r^2 > 0.999) from 5 to 2000 ng ml^{-1} using 1/x weighting. All HPLC runs had a total of 9 quality control samples, including 3 low (10 ng ml^{-1}), medium (150 ng ml^{-1}), and high (900 ng ml^{-1}). Intra and inter-day variability was less than 8%. Millenium Chromatography Software Version 1.1 (Waters, Mississauga, ON, Canada) or EZChrom Software Version 3.0 (Shimadzu Analytical Instruments Division, Kyoto, Japan) was used to integrate the peaks and process the chromatography.

2.9 Gross Morphological and Histopathological Assessment of Short- and Long-term Administration of P407 Experimentation

2.9.1 Protocol

Rats were randomly assigned to either a test group which received 1 gm kg^{-1}, P407, ip given as a one-time administration (n=2) or every 96 h for 4 (n=3), 6 (n=3), and 9 (n=3) wk. Control rats were similarily followed as test rats using saline ip over 9 weeks. At 48 and 96 h after the initial administration of P407, rats

were anesthetized with ether to enable blood collection (~ 200 µl) via the tail clip method.

2.9.2 Gross Morphological Postmortem Examination

Upon completion of the study period all rats were submitted to Health Sciences Laboratory Animal Services (HSLAS, University of Alberta, AB, Canada.) for pathological assessment. A gross postmortem morphological examination included an overall assessment of the entire body including skin, eyes, thorax, respiratory tract, gastrointestinal tract, peripheral vasculature, heart, spleen, pancreas, liver, adrenals, urinary tract, reproductive system, musculoskeletal system, brain and pituitary gland. Any abnormalities were noted including alterations in organ size, color, shape, structure, absesses and tumors. Lipid accumulation was characterized by variably sized, but usually, large single or multiple clear circular areas in the cytoplasm of affected cells. Lastly, if any abnormalities were noted in the gross morphological examination a histopathological examination was performed.

2.9.3 Histopathological Examination

The histopathological examination included removal and trimming of sections from select tissue which were processed routinely into paraffin blocks, sectioned at 5 microns and stained with hematoxylin and eosin. The excised tissues were fixed in 10% neutral buffered formalin. Following fixation for a minimum of 24 hours they were trimmed into 3 mm slices and placed onto plastic processing cassettes. Tissue in cassettes were placed in a Fisher automatic tissue processor and infiltrated with paraffin in a series of steps as follows:

1. dehydration through a series of increasing concentrations of alcohol
2. preparation for paraffin infiltration by saturation with xylene, and
3. infiltration with parrafin.

Following paraffin infiltration, tissues were removed from the processor, taken out of the cassettes, and manually embedded in a block of paraffin using a Fisher embedding centre. Paraffin blocks containing tissues were placed on a Reichert-Jung microtome, and a 5 micron thick section was cut and floated in a water bath onto glass slides. Glass slides with attached tissue sections were dried and then stained with hematoxylin and eosin stain using standard techniques. All procedures were performed by a trained histology technician assisted by a registered Animal Health Technician. Histology slides were read and interpreted by a Board Certified Veterinary Pathologist.

2.10 Effects of Hyperlipidemia on IV, IP and PO Nifedipine in the Rat Experimentation

2.10.1 Protocol

The animals were administered nifedipine (6 mg kg^{-1}) from a 5 mg ml^{-1} solution by either a 4 min intravenous (iv) infusion, intraperitoneal (ip) injection or oral (po) gavage. The iv dose was injected slowly from 0 to 4 min through the jugular vein cannula prior to blood sampling at time 0 and 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180 and 240 min after start of infusion. Ip injection was administered with a 12.7 mm 26G needle at time 0 min followed by blood samples at 2, 5, 30, 90, 180, 240, 300, 360, and 420 min. Oral gavage was administered by a stainless steel oral feeding tube at time 0 min followed by

blood samples at 5, 15, 30, 90, 180, 240, 360, 480, and 600 min.Total plasma cholesterol samples were taken 5 min. prior to PK study. Triglycerides were not measured because nifedipine is bound to the cholesterol rich lipoproteins, HDL and LDL, as opposed to the triglyceride rich lipoprotein VLDL.

2.11 Protein Binding Experimentation

Normolipidemia and hyperlipidemia were obtained by administering either 1 ml saline or 1 mg kg^{-1} P407 ip to male Sprague Dawley rats (340 - 380 g, n = 6) as in section 2.5. Blood was removed from the rats, via jugular vein cannula, 48 h post P407 administration. The plasma from each rat was pooled into the appropriate normolipidemic (n=3) or hyperlipidemic (n=3) group. The pH of the plasma was determined and adjusted as necessary to 7.4 ± 0.2 with 0.01 M HCl. The plasma samples were spiked with a solution of nifedipine in methanol to produce a final plasma concentration of 1.5 μg ml^{-1}. The residual concentration of methanol in plasma was less than 1.5% v/v. The spiked plasma was then incubated at 37.0 °C ± 0.3 for 30 min. The ultrafiltration units (Amicon Micropartition System MPA-1 Starter Kit, Amicon Div., W.R. Grace & Co., Danvers, MA, USA) combined with a YMT membrane disc (Amicon, Inc., Beverly, MA, USA) were filled with 1 ml aliquots of plasma. Samples were centrifuged at 2500 g for 30 min to obtain approximately 200 – 400 μl of ultrafiltrate. The ultrafiltrate of the normolipidemic and hyperlipidemic plasma was collected and pooled (n=3 and n=4, respectively) in order to provide an appropriate sensitivity for analysis. After the first spin the remaining plasma in the ultrafiltration unit was removed and 1 ml of phosphate (Sørensens) buffer (1/15

M, pH 7.4) was added and centrifuged for 30 min at 2500 g. The ultrafiltrate was collected and pooled as before. Five consecutive washes with buffer were carried out. Unfiltered samples were assayed and used as standards to enable the determination of percent recovery of each filtered sample. Protein leakage was determined by both a visual inspection of ultrafiltrate and acetonitrile treated ultrafiltrate (1:1) respectively, at which point any yellow-colored, turbid and/or precipitated ultrafiltrate would be discarded. No samples needed to be discarded. All samples were extracted and assayed on the day of the experiment. Unfiltered samples were assayed and used as standards for determination of fraction unbound in plasma of each filtered sample. Fraction unbound in plasma was calculated from f_{up} = (U1 + W1 + W2 + W3 + W4 + W5) / C_s, where U1 ,W1 , W2 , W3 , W4 and W5 are the total concentration of nifedipine in ultrafiltrate of the initial plasma spin, first, second, third, fourth and fifth buffer wash, respectively, and C_s is the total concentration of nifedipine in the unfiltered samples. The entire procedure was performed in a sodium lamp illuminated room to prevent nifedipine degradation.

Non-specific binding of nifedipine to the ultrafiltration unit and membrane was determined prior to the protein binding study to ensure complete recovery of nifedipine. Nifedipine was dissolved in methanol (0.10 mg ml^{-1}) and spiked into protein-free phosphate (Sørensens) buffer (1500 ng ml^{-1}). Aliquots of 1 ml were placed into ultrafiltration units. Three consecutive washes were performed on each ultrafiltration unit to determine the number of washes needed to achieve

complete recovery of nifedipine. After completion of washes the resulting ultrafiltrate and membranes were collected and assayed for nifedipine.

2.12 Inhibition of P407-Induced Hyperlipidemia using HMG CoA Reductase Inhibitors, Atorvastatin and Lovastatin Experimentation

2.12.1 Atorvastatin and Lovastatin Dosing

Stainless steel oral gavage tubes were used to administer lovastatin and atorvastatin suspensions. All suspensions were vortexed immediately prior to administration. Control rats that did not receive atorvastatin or lovastatin were administered 600 - 800 μl of plain methylcellulose suspension.

2.12.2 Protocol

To evaluate the inhibition of P407-induced hyperlipidemia two HMG CoA reductase inhibitors, atorvastatin and lovastatin, were administered via 3 different dosing schedules (A, B and C). Dosing schedule A (75 mg kg^{-1} dosing at 24 and 3 h prior to and 24 h post P407 administration), B (37.5 mg kg^{-1} dosing at 24, 12 and 3 h prior to and 12 and 24 h post P407 administration) and C (75 mg kg^{-1}dosing at 3 h prior to and 24 h post P407 administration). Twenty-one rats were divided into 7 groups, 6 groups receiving either atorvastatin or lovastatin using dosing schedule A, B or C and the P407 group, using no HMG CoA reductase treatment.

Baseline measurements of total plasma cholesterol and triglycerides were taken about 15 min prior to the first administration of atorvastatin, lovastatin or methylcellulose administration via the tail clip method. At the end of the study

period, 48 h post P407 administration, total plasma cholesterol and triglyceride levels were measured.

2.16 Nifedipine Dose-Response Experimentation

2.16.1 Protocol

Nifedipine doses of 0.05 (n = 3), 0.1 (n = 3) or 0.3 (n = 2) mg kg^{-1} were obtained from a 0.5, 1, and 3 and mg ml^{-1} nifedipine solution, respectively, to ensure equal volumes of each dose, which was administered as a 30 sec iv infusion through the jugular vein cannula.

2.16.2 Analysis

Relationships between concentration and percent change MAP and HR were determined using data from each individual rat following all doses from time 0 to 30 min. Hills equation, $E = E_{max} * C_n / EC_{50n} + C_n$ (9), was used to describe the concentration-effect curve for percent change in MAP, where E is the effect (% change in MAP), E_{max} is the maximum reduction in MAP, C is the nifedipine plasma concentration, EC_{50} is the concentration at 50% of maximum effect and n is the Hill coefficient.

2.17 Sodium Pentobarbital Experimentation

2.17.1 Protocol

The effects of pentobarbital sodium-induced anesthesia on MAP and HR were determined in normolipidemic (n = 3) and hyperlipidemic (n = 3) rats. The effects of an initial dose were studied from 30 to 60 min after administration of pentobarbital-sodium (65 mg kg^{-1}, ip), at which point a maintenance dose (20 mg kg^{-1}), was required. The hemodynamic effects of the maintenance dose were

determined 15 min following administration of the maintenance dose. The study period was from 30 to 60 min because the surgical preparation required 30 min.

2.18 Pharmacokinetic and Pharmacodynamic (PK-PD) Evaluation of Nifedipine in Hyperlipidemic and Atorvastatin Treated Hyperlipidemic Rat Experimentation

2.18.1 Atorvastatin

Three repeated oral doses of atorvastatin, 75 mg kg^{-1} dosing at 72, 48 and 24 h prior to the PK-PD study, were administered by a stainless steel oral gavage tube. The 48 h atorvastatin dose was administered 3 h prior to P407 administration. Rats that did not require atorvastatin were administered 800 µl of plain methylcellulose at the similar dosing schedule as atorvastatin.

2.18.2 Protocol

The rats were grouped as follows: (i) control or normolipidemic (NL) (n = 7), (ii) hyperlipidemic (HL) (n = 6), (iii) atorvastatin treated hyperlipidemic (HLA) (n = 8) and (iv) atorvastatin alone (A) (n = 6). Baseline MAP and HR were taken in triplicate immediately following completion of surgical procedures. No longer than 2 minutes after, nifedipine (1 mg kg^{-1}) was administered as a 30 sec iv infusion through the jugular vein cannula. PD measurements were taken about 5 - 20 sec prior to blood sampling. Blood samples of 150 - 250 µl were drawn at 0, 2, 5, 10, 15, 30, 45, 60, 90, and 120 min. An additional PD measurement was taken at 20 min. The carotid artery cannula was cleared of blood 15 sec prior to blood pressure measurement. The level of anesthesia throughout the study was monitored by the toe pinch (pedal) reflex. A maintenance dose of pentobarbital

sodium (20 mg kg^{-1}) was required at 60 - 75 min following the initial dose. Rats were kept warm by means of a wool blanket and a heating lamp.

2.19 Pharmacokinetic Analysis

PK parameters were determined by WINNONLIN Standard Edition Version 1.0 (Scientific Consulting Inc., Apex, NC, U.S.A). Non-compartmental methods were used to describe the disposition of nifedipine, in which no assumption for a specific compartment model is required. The linear trapezoidal method was used to calculate area under the concentration-time curve ($AUC_{0\text{-}last}$) and area under the first moment concentration-time curve ($AUMC_{0\text{-}last}$) from time 0 to the last measurable concentration. The area under the concentration-time curve and area under the first moment curve from time 0 to infinity were calculated from $AUC_{0\text{-}\infty} = AUC_{0\text{-}last} + (C_{last}/\lambda)$ and $AUMC_{0\text{-}\infty} = AUMC_{0\text{-}last} + [t_{last} \bullet (C_{last}/\lambda)] + (C_{last}/\lambda^2)$, respectively, where C_{last} is the last measureable concentration, t_{last} is the time of the last measurable concentration and λ is elimination rate constant. AUC for total ($AUC_{T(0\text{-}30min)}$) and unbound ($AUC_{U(0\text{-}30min)}$) concentrations from 0 to 30 min were also calculated to enable comparisons with area under the effect-time curves ($AUEC_{(0\text{-}30min)}$). Comparisons of $AUEC_{(0\text{-}30min)}$ with $AUC_{0\text{-}\infty}$ were not appropriate, because MAP was only measured from 0 to 30 min. $AUC_{U(0\text{-}30)}$ for the NL and HL groups were calculated by the following equation; $AUC_{U(0\text{-}30min)} = f_{up} \bullet AUC_{T(0\text{-}30min)}$. The average f_{up} for NL and HL plasma were 0.035 and 0.024, respectively, which were found in a previous study (see section 4.1) using P407-induced hyperlipidemic and normolipidemic rat plasma.

Total body clearance (CL_{TB}), and ip ($CL/F_{(ip)}$) and po clearance ($CL/F_{(po)}$) were calculated as Dose / $AUC_{0-\infty}$. The λ was estimated by linear regression using the last 3 - 6 concentration data points. Vd_{ss} was calculated as Vd_{ss} = (Dose / $AUC_{0-\infty}$) • ($AUMC_{0-\infty}$ / $AUC_{0-\infty}$). Absolute bioavailability (F) and fraction of drug escaping hepatic elimination (F_L) were calculated using the ratio of the mean $AUC_{0-\infty}$ after po or ip doses over iv $AUC_{0-\infty}$, respectively.

2.20 Pharmacodynamic Analysis

Pharmacodynamic analysis included maximum % decrease in MAP, $AUEC_{(0\text{-}30min)}$ and pharmacological half-life (pharmacological t½). The maximum % decrease in MAP was interpreted from the effect-time curves, all of which occurred at 2 min. $AUEC_{(0\text{-}30min)}$ was calculated by the linear trapezoidal method. The λ was calculated by linear regression of the time versus logarithmic effect curve using absolute values, and the pharmacological t½ was found as $0.693/\lambda$.

2.21 Pharmacokinetic - Pharmacodynamic Analysis

The percent change in MAP was related to the nifedipine plasma concentration by means of the linear model according to E = m * C + b, where E is the measured effect (% change MAP), C is nifedipine plasma concentration in ng ml^{-1}, m is the slope and b is the y intercept.

2.22 Statistical comparisons

Statistical comparisons were carried out on all data by either a one-way ANOVA with Duncan New Multiple Range Test for multiple comparisons or independent t-tests. Linear regressions were carried out using the simple linear regression method. All pharmacokinetic parameters, except t_{max}, were log

transformed prior to statistical analysis. A $p < 0.05$ was considered statistically significant. Data are reported as mean $\pm$ SD, unless stated otherwise.

Chapter 3

Gross Morphological and Histopathological Postmortem Assessment of Acute and Chronic Administration of P407

3.1 Results

No significant differences were found in initial weights of rats (198-242 g). Rats in each group gained approximately 5 - 6 g day^{-1}, which is the average weight gain of a normal healthy rat (181). The final body weights of test rats after 4, 6 and 9 wk of P407 administration and control rats after 9 wk of saline administration were 426 ± 31, 475 ± 19, 613 ± 11 and 638 ± 24, respectively. Notably, there were no significant differences between final weights of test and control rats after 9 wk. Total plasma cholesterol levels were significantly elevated 48 and 96 h after P407 administration in all test rats (9.40 ± 1.69 and 7.29 ± 1.64, respectively), in contrast to the normolipidemic control rats (1.07 ± 0.30 and 1.30 ± 0.14, respectively). Gross morphological examinations reported that all rats after one time administration and 4 and 6 wk administration of P407 were hyperlipidemic but otherwise healthy, with the exception of one rat at 6 wk. This rat had early lipid accumulation of infiltrating macrophages within the intimal cells of the aorta, possibly resembling a foam cell. Test rats after 9 wk of P407 administration had extensive lipid deposition in the cytoplasm of hepatocytes, renal cortical and adrenocortical cells, and macrophages within splenic sinusoids and pulmonary alveoli. However, intimal cells of the aorta were unaffected. The adrenal glands in all the 9 wk test rats were enlarged. Internal bleeding was not observed in any test rats. Throughout the entire duration of the study, one rat died within the 6 wk group.

3.2 Discussion

P407-induced hyperlipidemia appears to be a convenient and inexpensive hyperlipidemic rat model (15,81), however this model is relatively new and has not been fully characterized. Although P407 has been noted to be a non-toxic polymer (78,87,88,182), a postmorten gross morphological and histopathological study was performed to evaluate overall toxicological and pathological effects of ip administration of 1 g kg^{-1} P407 every 96 h, for short- and long-term administration. Total plasma cholesterol levels were elevated 48 h post P407 administration and remained elevated by 96 h, which has also been observed in previous studies (80,81). The development of foam cells, fat-filled macrophages in the intimal layer of smooth muscle cells (183) in one of the 6 wk test rats may resemble an early stage of an atherosclerotic lesion. Generally, rats are very resistant to atherosclerosis due to efficient lipoprotein metabolism, higher concentrations of HDL, and lower concentrations of LDL, an important factor since LDL cholesterol has been shown to be the major atherogenic lipoprotein (43,183,184). Regardless, in some rat models sustained hyperlipidemia has resulted in atherosclerosis (19,68). In the current study, the 9 wk test rats had lipid infiltration in numerous tissues, but otherwise these rats were healthy.

The most widely used criteria for measurement of hepatic toxicity in rats are a reduction in the rate of body weight gain, detection of gross and histological abnormalities in the relevant organs, changes in organ weights, and increases in mortality rate (185). In the present study, all treated rats gained weight as normal, and mortality rate was not increased. Although one rat died, the death

was later found by autopsy to be most likely due to gagging or choking on the bedding chips, that were found within the oral cavity and covering the larynx. In addition, liver size, color and weight were unaltered in all test rats. In comparison, other investigators have found that repeated ip administration of P407 at doses of 0.33 g kg^{-1} increases monocyte concentrations with no other toxicities, whereas 1.0 g kg^{-1} every 24 hours for 4 days causes a reduction in body weight, altered blood cell counts, and slight splenomegaly (89). To address this, the authors concluded that sequestration of lipid-containing macrophages in the red pulp of the spleen caused the splenomegaly, which was not surprising considering the large increases in plasma lipids (89). It appears that dosing P407 at 1 g kg^{-1} every 24 h is toxic. In contrast, dosing P407 1 g kg^{-1} every 96 h as in the present studies does not appear to have similar toxicities. Therefore, the P407-induced hyperlipidemic rat model appears to be a suitable hyperlipidemic model to study the effects of hyperlipidemia on the PK and PD of nifedipine after one time administration of P407 or up to 4 wk of administration every 96 h.

Chapter 4

The Effects of Hyperlipidemia on the PK of Nifedipine in the Rat

---

A version of this paper has been accepted for publication. L. A. Eliot[1], R. T. Foster[2] and F. Jamali[1] (1997) *Pharm. Res* . 1. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada. 2. Isotechnika, Edmonton, Alberta, Canada.

4.1 Results

Nifedipine was absorbed and eliminated rapidly so that the last collected samples generally contained no detectable concentration (Fig. 4-1).

P407-induced hyperlipidemia caused significant elevations of total plasma cholesterol (Table 4-1). This was associated with significant decreased CL_{TB} and consequently increased AUC of nifedipine (Table 4-1). Significant positive correlations in the hyperlipidemic rats were found between nifedipine AUC and total plasma cholesterol in the iv and po dosed rats ($r = 0.92$, $p = 0.028$, and $r = 0.90$, $p = 0.016$, respectively) but not following ip doses (Fig. 4-2). There was a significant prolongation of t½ following iv and ip doses. Half-life and λ was not measured after po doses due to excessive fluctuation in the elimination phase, therefore $AUC_{0-\infty}$ was not determined. The $AUC_{0\text{-last}}$ was used instead of $AUC_{0-\infty}$, to calculate $Cl/F_{(po)}$ and F. This is unlikely to introduce error because the concentrations at the last time point were nearly at the lowest limit of quantitation. Vd_{ss} was not significantly affected by hyperlipidemia. The route of administration did not significantly influence the observed effects of P407-induced hyperlipidemia. No differences in absolute bioavailability (F) were observed between the normolipidemic and hyperlipidemic rats.

The % recovery of nifedipine for the first, second and third ultrafiltration in protein-free phosphate buffer was 92.90 ± 4.74%, 107.8 ± 1.33% and 109.63 ± 1.25%, respectively. Therefore, it was necessary to wash the ultrafiltration units twice in order to ensure complete recovery. Nevertheless, five washes were used

for the plasma ultracentrifugation experiment. Nifedipine was undetectable after the fifth wash.

The f_{up} of nifedipine was significantly different between the normolipidemic (0.035 ± 0.002) and hyperlipidemic (0.024 ± 0.001) rats at a concentration of 1.5 μg ml^{-1}, which was representative of the maximum plasma concentration in vivo in po dosed rats. Since nifedipine has demonstrated concentration-independent protein binding within concentrations ranging from 0.1 μg ml^{-1} - 10 μg ml^{-1} (128,170) only one dose was necessary to assess the effects of hyperlipidemia on the f_{up} of nifedipine. Total plasma cholesterol levels of the normolipidemic and hyperlipidemic plasma used for the protein binding study were 0.82 ± 0.35 and 12.83 ± 1.4 mmol L^{-1}, respectively.

4.2 Discussion

Nifedipine oral bioavailability in the rat (169) and human (165,186) is approximately 0.45 - 0.60 and 0.40 - 0.68, respectively. This low bioavailability has been attributed to a first pass metabolism rather than incomplete absorption (187,188). Recently, it has been shown that the gastrointestinal tract substantially contributes to pre-systemic elimination of nifedipine in the rat (169). Therefore to differentiate between the gastrointestinal and hepatic presystemic elimination of nifedipine both ip and po doses were administered and compared. Our observation that AUC of nifedipine following ip doses is almost equal to that observed after iv administration (Table 4-1) further supports involvement of the gut rather than liver in the presystemic clearance of the drug.

Hyperlipidemia was induced in our rats using P407, which was adapted from a previously characterized model (80). Convenience and ability to replicate the human condition are two important issues when choosing an animal model. Although lipoprotein metabolism is substantially different in the rat than the human, the rat is extensively used for lipid research (26). The plasma lipoprotein distribution that comprises total plasma cholesterol in rats does not resemble that of humans. Rats carry 60 - 80% of plasma cholesterol in HDL, whereas humans carry 60 - 70% in LDL and only 20 - 30% in HDL (14). Perhaps an increase in protein binding resulting in a decrease in f_{up} occurs despite the differing lipoprotein distributions because nifedipine binds to both LDL and HDL (129). Furthermore, hyperlipidemia alters the pharmacokinetics of cyclosporine A similarly in the rat and human (103,108,109,189). Although nifedipine and cyclosporine A have different physicochemical properties, comparisons of the effects of hyperlipidemia on their PK parameters are important because they both associate with the major cholesterol containing lipoproteins, HDL and LDL.

The magnitude of the inhibitory effect of hyperlipidemia on CL_{TB} of nifedipine was independent of the route of administration. Since the effect was not greater after po doses as compared with iv and ip administrations, it is reasonable to suggest that the first-pass gut metabolism might not have been differentially affected by hyperlipidemia. Therefore, a reduced systemic clearance is a plausible explanation for the observed P407-induced reduced nifedipine clearance. An increased binding to plasma lipoproteins might have caused this effect on nifedipine, a drug with low hepatic extraction in the rat (169).

It has been shown that lipoproteins interact with a number of lipophilic drugs and hyperlipidemia increases the binding capacity of the lipoproteins (41,97,107). In vitro studies have shown that nifedipine binds to albumin, AAG and lipoproteins with a f_{up} of 0.01 - 0.09 (129,171). Our data indicates that hyperlipidemia results in a significant 31% decrease in f_{up} of nifedipine in plasma (Table 4-1). This is similar to previous reports suggestive of an inverse correlation between lipoprotein concentrations and f_{up} for cyclosporine A (96), fentanyl (100), imipramine (98) and isradipine (95). In addition to CL_{TB}, Vd_{ss} may be altered in response to variations in the f_{up}. This is only when one assumes that the fraction unbound in the tissue (f_{ut}), and tissue volume (V_t) or plasma volume (V_p) do not change. The unchanged Vd_{ss} observed in our study may be indicative of either no changes in f_{ut} and increases in V_t or proportional changes in both f_{up} and f_{ut}, resulting in an unchanged Vd_{ss}.

Hyperlipidemia caused only a 31% reduction in f_{up} and approximately a 40% reduction in CL_{TB} regardless of the route of administration (Table 4-1). The variability in the reduction of f_{up} does not account for the complete variability in reduction of Cl_{TB} since reductions in Cl_{TB} range to a much larger extent than the reduction in f_{up}. Hence, the effect of hyperlipidemia on the pharmacokinetics of nifedipine may be completely explained by altered binding of the drug to plasma proteins. Nevertheless, since the effect on CL_{TB} somehow exceeds the observed increase in the extent of binding, the possibility of other mechanism(s) such as a decrease in hepatic uptake of lipoproteins caused by a down-regulation in LDL receptors (10,34) and/or a reduced CLu_{int} by a decrease in CYP450 content or

activity (137,141,142) cannot be ruled out. In addition, it has been suggested that the regulatory mechanisms of cytochrome P450 and hepatic p-glycoprotein are overlapping (190). A decrease in CYP450 may produce a decrease in p-glycoprotein in the liver and decrease hepatic uptake of nifedipine. Also, because p-glycoprotein is an ATP driven transporter an inactivation of p-glycoprotein ATPase, due to the presence of lipids (191) may also decrease hepatic uptake of nifedipine.

The increases in t½ in the hyperlipidemic state may not be explained by the decreases in f_{up}, since the t½ of highly distributed drugs are mainly dependent on changes in tissue binding as opposed to plasma binding. The observed increases in t½ in the HL group may substantiate suggestions of a decrease in CLu_{int} mentioned in the previous paragraph. However, an increase in V_t or a decrease in f_{ut} may also contribute.

4.3 Conclusions

P407-induced hyperlipidemia appears to produce a decrease in systemic clearance of nifedipine. A decrease in unbound fraction of nifedipine in hyperlipidemic plasma may, at least in part, be responsible. A decrease in hepatic uptake of lipoproteins, cytochrome P450 and p-glycoprotein ATPase activity cannot be ruled out (192).

Table 4-I. Comparison of pharmacokinetic data of nifedipine (6 mg kg^{-1}) in normolipidemic and hyperlipidemic rats administered via iv, ip and po routes (n = 5 or 6 rats per group). Parameters are expressed as arithmetic means ± SD. Superscript [a] indicates significantly different from other routes of administration and [b] indicates significantly different from normal. n.d. Not determined.

	iv		*ip*		*po*	
	NL	***HL***	***NL***	***HL***	***NL***	***HL***
Total Plasma Cholesterol (mmol L^{-1})	1.52 ± 0.13	9.17[b] ± 1.71	1.38 ± 0.39	9.14[b] ± 2.28	1.36 ± 0.20	8.10[b] ± 0.41
$AUC_{0\text{-last}}$ (ug min ml^{-1})	390 ± 49	639[b] ± 126	358 ± 59	638[b] ± 149	174[a] ± 46	297[b] ± 21
$AUC_{0\text{-}\infty}$ (ug min ml^{-1})	392 ± 49	647[b] ± 135	359 ± 58	640[b] ± 149	n.d.	n.d.
C_{max} (µg ml^{-1})			5.24 ± 2.06	9.48[b] ± 1.84	0.92 ± 0.22	1.55[b] ± 0.63
t_{max} (min)			23 ± 12	13 ± 13	45 ± 35	30 ± 0
Vd_{ss} (ml kg^{-1})	429 ± 60	386 ± 44				
CL_{TB} (ml $min^{-1}kg^{-1}$)	15.5 ± 1.9	9.5[b] ± 1.6				
$Cl/F_{(ip\ and\ po)}$ (ml $min^{-1}kg^{-1}$)			17.1 ± 2.9	9.8[b] ± 2.3	35.0 4.9 ±	20.6[b] 3.3 ±
$t_{½}$ (min)	19 ± 4.0	31[b] ± 8.0	24 ± 5	36[b] ± 12	n.d.	n.d.
F	1.0	1.0	0.91	0.99	0.45	0.46

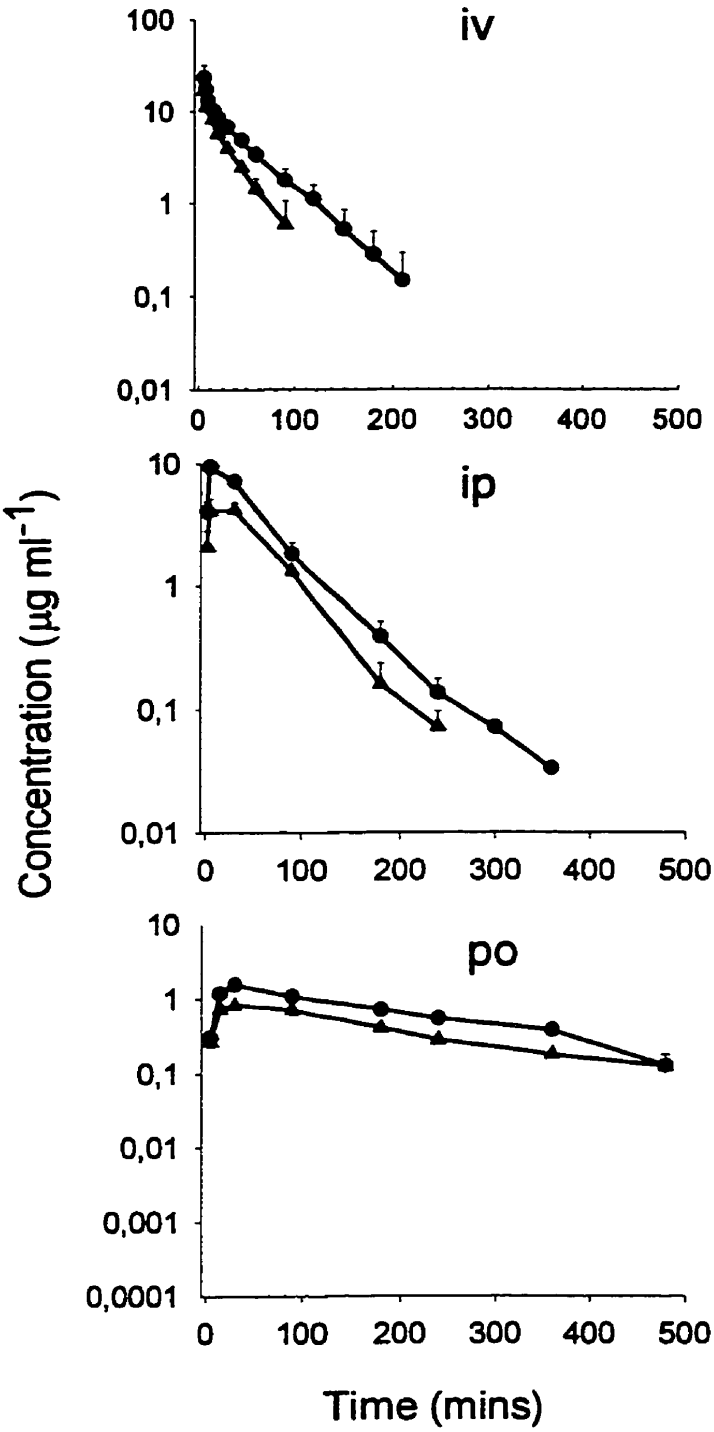

Fig. 4-1. Concentration-time profiles for iv, ip and po administration of nifedipine (6 mg kg^{-1}) in hyperlipidemic ● and ▲ normolipidemic rats. Data is presented as arithmetic mean ± SD.

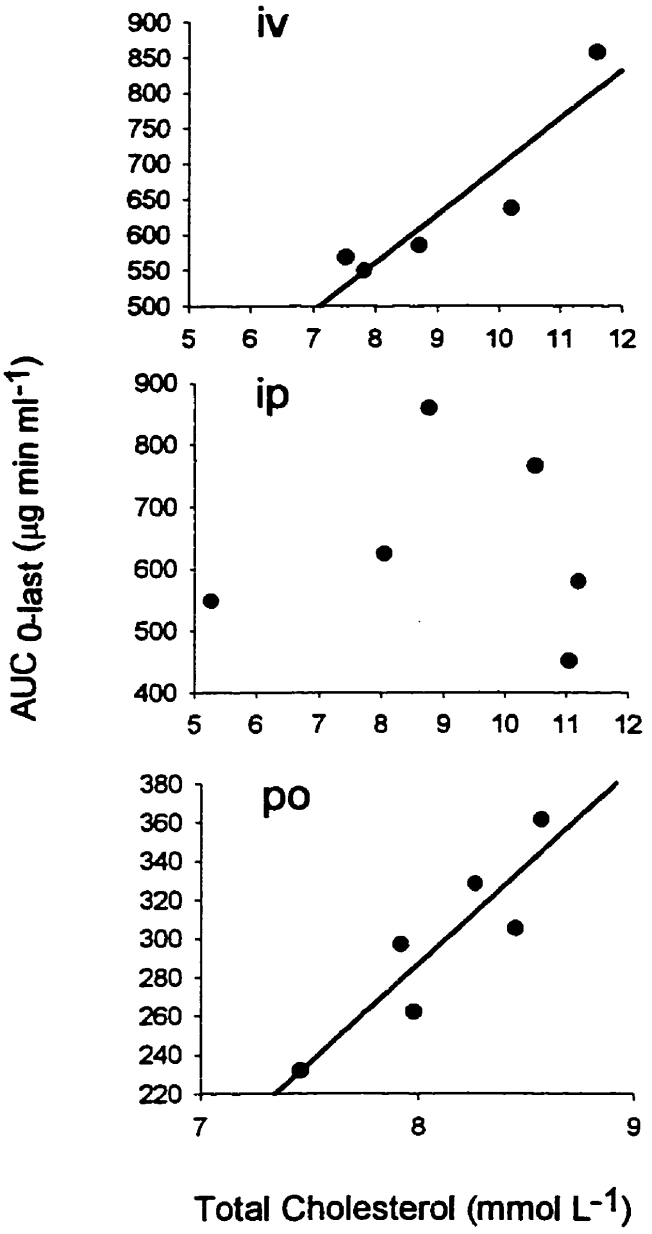

Fig. 4-2. Correlation of nifedipine AUC and total plasma cholesterol concentration after iv ($r = 0.92$, $p = 0.028$), ip ($r = -0.16$, $p = 0.80$) and po ($r = 0.90$, $p < 0.016$) administration.

Chapter 5

Cholesterol Lowering in P407-Induced Hyperlipidemic Rats by Atorvastatin and Lovastatin is Dependent on the Dosing Schedule, Not on the Therapeutic Entity.

5.1 Results

Total plasma cholesterol was significantly increased by 8.4 fold ($p < 0.001$) 48 h post administration of P407 in the group receiving only P407 (Table 5-1). The total plasma cholesterol lowering ability of atorvastatin and lovastatin were similar for each dosing schedule (Fig. 5-1). Atorvastatin and lovastatin administration using dosing schedule A (75 mg kg^{-1} dosing at 24 and 3 h prior to and 24 h post P407 administration) was the only regime which significantly inhibited P407-induced increases in total plasma cholesterol levels. Atorvastatin and lovastatin inhibited increases in total plasma cholesterol levels by 58% ($p < 0.001$) and 56% ($p < 0.001$) in comparison to the P407 group, respectively. However, total plasma cholesterol remained significantly increased above baseline measurements by 2.0 ($p < 0.001$) and 2.5 ($p < 0.001$) fold, with both inhibitors using dosing schedule A.

Total plasma cholesterol was increased 8.3 ($p < 0.001$) and 9.1 fold ($p < 0.001$) using dosing schedule C (75 mg kg^{-1} dosing at 24 and 3 h prior to and 24 h post administration) for atorvastatin and lovastatin, respectively. These increases were similar to those produced by the P407 group.

Interestingly, dosing schedule B (37.5 mg kg^{-1} dosing at 24, 12 and 3 h prior to and 12 and 24 h post P407 administration) increased total plasma cholesterol levels 12.8 ($p < 0.001$) and 11 fold ($p < 0.001$) above baseline using atorvastatin and lovastatin, respectively, which is 32 - 52% ($p < 0.05$) higher than the total plasma cholesterol levels in the P407 group. (Fig. 5-1).

Triglyceride levels were increased from 1.18 ± 0.08 to greater than 4.23 mmol L^{-1}, however the exact values were not quantified due to assay limitations. The triglyceride levels were greater than the highest quantifiable amount and there was not enough plasma remaining to perform serial dilutions.

5.2 Discussion

Previously it has been shown that P407-induced hyperlipidemia decreases CL_{TB} and f_{up} of nifedipine (193), therefore, it is of interest to evaluate the effects of lowering total plasma cholesterol levels, in an attempt to normalize the PK of nifedipine.

In the last decade a new class of drugs, HMG CoA reductase inhibitors, have been introduced to lower serum cholesterol and triglycerides. Today there are 5 HMG CoA reductase inhibitors on the market; lovastatin, simvastatin, pravastatin, fluvastatin and atorvastatin. The mechanism of action of this drug class involves alteration of cholesterol biosynthesis and metabolism. In many species, including rat and human, lovastatin and atorvastatin are potent inhibitors of HMG CoA reductase, producing a decrease in the hepatic pool of cholesterol. As a result, a decrease in the formation of VLDL (3,4) and an upregulation of LDL receptors occurs, which leads to a decrease in total plasma cholesterol. The exact contributions of these mechanisms to the overall lowering of total plasma cholesterol have not been determined and may be species dependent.

The hypolipidemic effects of HMG CoA reductase inhibitors in the rat has produced a body of conflicting evidence, with reports showing a decrease (15,81,194,195), increase (194,196) or unchanged (194,197-200) total plasma

cholesterol levels. Perhaps these results are dependent on the particular model used, therapeutic entity, and dosing regime of the therapeutic entity. In the present study a decrease, increase and unchanged total plasma cholesterol level was observed depending on the dosing schedule, but not on the inhibitor that was used.

Dosing schedule A (75 mg kg^{-1} dosing at 24 and 3 h prior to and 24 h post P407 administration) was designed to adhere to the study protocol which completely inhibited P407-induced hyperlipidemia using lovastatin 75 mg kg^{-1} dosing at 24 and 1 h prior to and 24 h post P407 administration (81). In accordance, significant decreases in total plasma cholesterol were observed, although complete suppression did not occur. However, using dosing schedule A with either inhibitor decreased total plasma cholesterol to a greater extent than reported for pravastatin, dosing 75 mg kg^{-1} at 24 and 0 h prior to and 24 h post P407 administration (15). The authors explained that the administration of the second dose of pravastatin at time of injection (0 h) of P407, rather than 1 hr prior, may be the cause for the minimal inhibition. Accordingly, it has been suggested that the efficacy of lowering total plasma cholesterol with an HMG CoA reductase inhibitor in P407-induced hyperlipidemia is dependent on dose and timing of administration with respect to P407 administration. To account for this, the present study allowed ample time (3 h) for absorption of atorvastatin and lovastatin, prior to P407 administration.

Dosing schedule B (37.5 mg kg^{-1} dosing at 24, 12 and 3 h prior to and 12 and 24 h post P407 administration) was designed to suppress the latent

induction of HMG CoA reductase activity reported to occur 12 - 20 h after administration of the HMG CoA reductase inhibitor (197). Short-term and chronic administration of HMG CoA reductase inhibitors produced an increase in enzyme expression due to a cellular adaptation of the HMG CoA reductase enzyme (201). However, this increased enzyme expression is inconsequential as long as the drug's concentration (HMG CoA reductase inhibitor) remains above the minimum inhibitory level (202), after which the activity is expressed and cholesterol genesis increases to rates above control values (197,203,204). In contrast to expectations, the percent change in total plasma cholesterol was significantly higher than the P407 group, which may be attributed to the dual effects of HMG CoA reductase induction and a sub-optimal concentration of both atorvastatin and lovastatin. Furthermore, the suboptimal concentration of atorvastatin and lovastatin may arise from a decrease in bioavailability in twice daily dosing, since feeding occurs in conjunction with the second dose.

Dosing schedule C (75 mg kg^{-1} dosing at 3 h prior to and 24 h post P407 administration) was designed to determine the importance of the 24 h dose of HMG CoA reductase inhibitor prior to P407 administration. The lack of cholesterol lowering suggests that the 24 h prior to P407 statin dose is necessary to inhibit P407-induced hyperlipidemia, which corroborates previous findings (15).

The similar reductions in total plasma cholesterol occurring after lovastatin and atorvastatin dosing were not expected, since in vitro studies with rat liver microsomes (205,206) or hepatocytes (207) have shown that atorvastatin is a

more potent hypolipidemic agent than lovastatin. These in vitro results, in turn, have correlated well with in vivo PD studies in many species including rat, guinea pig, rabbit and human (200). As well, the PK profile of atorvastatin is more prolonged than lovastatin in plasma, however the extensive metabolic profile and multitude of PD effects have, as yet, precluded the development of functional PK-PD models for HMG Co A reductase inhibitors (56,201). Currently, these findings have not indicated any qualitative differences in effects on total plasma cholesterol levels between the HMG CoA reductase inhibitors tested, notwithstanding the respective effects on total plasma triglyceride levels, which were not assessed in this study.

It is currently recognized that the normal rat has lower total plasma cholesterol levels and a different lipoprotein distribution than human (15). Also, HMG CoA reductase inhibitors may increase rather than decrease total plasma cholesterol in the normal rat (196), although the majority of studies show no observable changes (197-199). These overt limitations for lipid research in the normolipidemic rat may be overcome by producing a state of hyperlipidemia similar to that seen in human (15,195). P407 elevates total plasma cholesterol to levels comparable to the hyperlipidemic state in human, which can be inhibited by HMG CoA reductase inhibitors (15,81). Furthermore, the lipoprotein distribution may be altered to resemble a hyperlipidemic condition in the human more closely, although this was not determined in the current study (15). Despite the limitations in the normal rat, the P407-induced hyperlipidemic rat may be a

suitable model to study the effects of hyperlipidemia and subsequent treatment of, on the PK and PD of drugs.

5.3 Conclusions

P407-induced hyperlipidemia increases total plasma cholesterol, which may be partially inhibited by HMG CoA reductase inhibitors. The dosing schedule, rather than the particular HMG CoA reductase inhibitor, atorvastatin or lovastatin, is crucial to the inhibition P407-induced hyperlipidemia.

Table 5-1. Total plasma cholesterol concentrations (mmol L^{-1}, mean ± SD) at time zero (naive rats, prior to P407 and statin administration) and 48 h after administration of P407 and HMG Co A reductase inhibitors (statins). Significant differences ($p < 0.05$) between total plasma cholesterol at 0 h and 48 h are represented by [a].

Treatment	Dosing Schedule	Total Cholesterol at 0 h (prior to P407 and statin administration)	Total Cholesterol at 48 h post-P407 administration and 24 h post statin treatments
P407		0.92 ± 0.07	8.61 ± 0.03[a]
P407 + Atorvastatin	A: 75 mg kg^{-1} at 24 and 3 h prior to and 24 h post P407	1.19 ± 0.19	3.60 ± 0.27[a]
P407 + Lovastatin		1.10 ± 0.15	3.80 ± 0.87[a]
P407 + Atorvastatin	B: 37.5 mg kg^{-1} at 24, 12, and 3 h prior to and 12 and 24 h post P407	0.63 ± 0.12	8.72 ± 0.38[a]
P407 + Lovastatin		0.71 ± 0.17	8.51 ± 0.39[a]
P407 + Atorvastatin	C: 75 mg kg^{-1} at 3 h prior to and 24 h post P407	0.92 ± 0.10	8.57 ± 0.44[a]
P407 + Lovastatin		0.87 ± 0.13	8.83 ± 0.65[a]

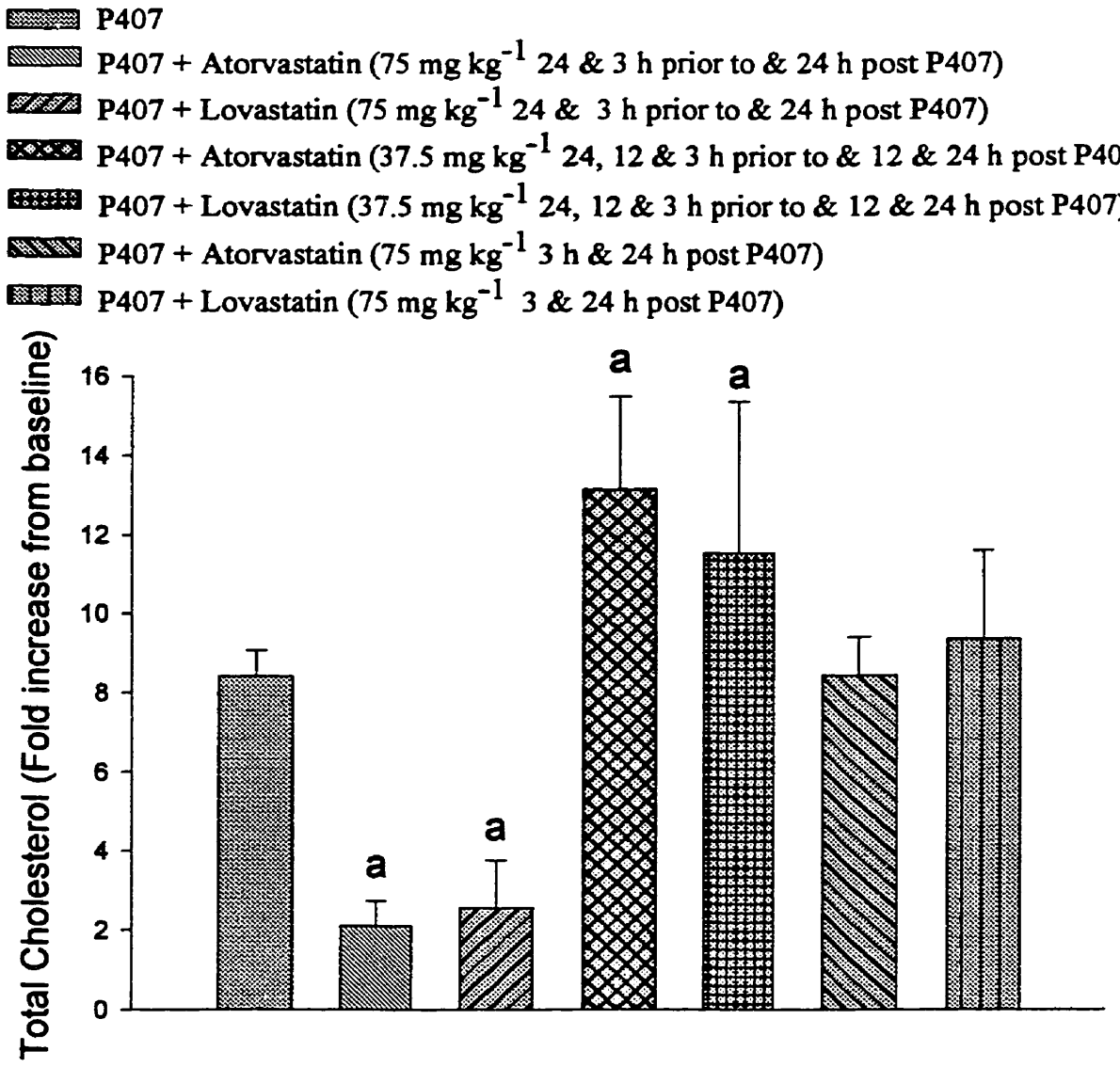

Fig. 5-1. Fold increase in total plasma cholesterol concentration from baseline (naive rats, prior to P407 and statin treatment). Significant differences from the group receiving only P407 aredenoted by a.

Chapter 6

Pharmacokinetics and Pharmacodynamics of Nifedipine in Atorvastatin Treated Hyperlipidemia in the Anesthetized Rat

---

A version of this paper has been submitted for publication. L. A. Eliot and F. Jamali (1998) Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

6.1 Results

6.1.1 Effect of Pentobarbital Sodium-Induced Anesthesia on Baseline MAP and HR.

The effects of pentobarbital sodium-induced anesthesia (65 mg kg^{-1}, ip) on MAP and HR were studied in NL and HL rats (Fig. 6-1). Baseline MAP and HR were similar in the NL and HL groups. MAP gradually increased, from 0.2 and 1.5% to approximately 8% at 30 min in both NL and HL groups, respectively. Unlike MAP, HR displayed no consistent change with a substantial degree of inter- and intra-animal variability throughout the duration of the study. Maintenance doses of pentobarbital sodium (20 mg kg^{-1}) were required at 30 min, determined by a positive response to the pedal reflex, which produced a decrease in MAP in all rats, with the exception of one rat in the NL group, which remained unaltered. The averaged decrease in MAP was 9 ± 2 and 6 ± 5% in NL and HL groups, respectively. In addition, HR decreased by 5 ± 5 and 6 ± 4% in the NL and HL rats, respectively, indicating a pentobarbital sodium-induced bradycardia. No significant differences in MAP and HR between the NL and HL groups were detected throughout the entire duration of the study.

6.1.2 Dose-Response

Fig. 6-2 depicts the nifedipine concentration-dependent and -independent reduction in MAP and HR, respectively. Therefore MAP, but not HR, was used to assess the pharmacological response nifedipine. The percent decrease in MAP was described by the sigmoidal E_{max} model (Please refer to section 2.16.2). E_{max}, EC_{50} and n were estimated to be -46 ± 5%, 435 ± 72 ng ml^{-1} and 2 ± 0.4,

respectively. The 0.05 and 0.1 mg kg^{-1} doses of nifedipine generally resulted in concentrations within the log-linear portion of the concentration-effect curve for the study period. The majority of other concentrations following the 0.3 mg kg^{-1} dose were in the plateau (E_{max}) phase of the effect-concentration curve.

6.1.3 Comparison of nifedipine PK-PD in NL, HL, HLA, and ATV groups

Total plasma cholesterol concentrations were 1.4 ± 0.3 and 1.4 ± 0.2 mmol L^{-1} in the NL and ATV groups, respectively. P407-induced hyperlipidemia significantly increased total plasma cholesterol to 12.4 ± 1.1 mmol L^{-1} in comparison to the NL and ATV groups. Therapeutic intervention with atorvastatin in the HLA group produced variable decreases in total plasma cholesterol, ranging from 0 to 70%, resulting in total plasma cholesterol levels of 9.2 ± 3.5 mmol L^{-1}, significantly lower when compared to the HL group and significantly higher than the NL and ATV groups.

$AUC_{T(0-\infty)}$ was significantly increased by 66% in the HL group compared to the NL and ATV groups (Table 6-1). Cholesterol lowering by atorvastatin treatment (HLA group) decreased the $AUC_{T(0-\infty)}$ so that it was not significantly different than the NL and ATV groups. A positive correlation between $AUC_{T(0-\infty)}$ and total plasma cholesterol levels was found ($r = 0.71$, $p < 0.0001$). Cl_{sys} of nifedipine was affected to a similar extent as $AUC_{T(0-\infty)}$. In addition, a significant inverse correlation ($r = 0.67$, $p = 0.004$) was found between Cl_{sys} and total plasma cholesterol (Fig. 6-3). Plasma nifedipine concentrations were similar in all groups during the first 15 min (Fig. 6-4), therefore $AUC_{T(0-30min)}$ was similar for the NL and HL groups. In contrast $AUC_{U(0-30min)}$ was significantly ($p < 0.001$) lower in the HL

group compared to the NL group. From 15 min onward the concentrations in the HL and HLA groups declined slower than the other groups (Fig. 6-4). Accordingly, the t½ of elimination was significantly increased by 85 and 35% in the HL and HLA group, respectively, compared to the NL group. Cholesterol lowering by atorvastatin treatment (HLA group) decreased t½ by 27% in the HLA group compared to the HL group, to the extent where t½ in the HLA group was not significantly different than in the NL and ATV groups. (Table 6-1). In addition, a positive correlation between t½ and total plasma cholesterol ($r = 0.76$, $p = 0.002$) was found (Fig. 6-5). No significant changes in Vd_{ss} were found for the HL, HLA and ATV groups in comparison to the NL group. ATV rats, however, exhibited a lower Vd_{ss} values than the HL and HLA rats.

There was a statistically insignificant decreasing trend of maximum percent change in MAP (12% in the HL and HLA groups compared to the NL and ATV groups) (Table 6-2). MAP increased rapidly approaching baseline in 30 min in the NL and A groups. As Fig. 6-6 illustrates, the return of MAP to baseline was slower in the HL group so that a significant difference in percent change MAP was observed at 30 min in comparison to the other groups. As a result, pharmacological t½ of nifedipine was increased 4 fold in the HL group compared to NL and A groups (Table 6-2). There was a significant and positive correlation between pharmacological and pharmacokinetic t½ ($r = 0.50$, $p = 0.011$).

Atorvastatin intervention (HLA group) produced a significant decrease in pharmacological t½ of 1.9 fold compared to HL group, nevertheless it remained significantly higher than the NL and ATV groups. A positive correlation between

pharmacological t½ and total plasma cholesterol ($r = 0.80$, $p < 0.001$) was found (Fig. 6-7). $AUEC_{(0\text{-}30min)}$ was not significantly increased in the HL group as compared with the NL group despite the increasing trend. Nifedipine plasma concentration and percent reduction in MAP was described by the linear model as illustrated in Fig. 6-8. The slopes (m) of the linear regression for effect-concentration curves were not different between the four groups.

6.2 Discussion

Nifedipine is a potent arterial vasodilator, which decreases systemic vascular resistance and blood pressure (175,208). Unfortunately, sympathetic stimulation, due to a reflex baroreceptor mechanism, is accomplished by tachycardia. Many studies in conscious and unconscious rats have found that nifedipine induces a concentration-dependent and -independent decrease in MAP (209) and HR (210), respectively. Within the doses used in this study (0.05, 0.1 and 0.3 mg kg^{-1}) nifedipine exhibited a sigmoidal plasma concentration-effect relationship (Fig. 6-2.A). At a dose of 0.1 mg kg^{-1} nifedipine concentrations ranged within the log-linear phase of the concentration-effect curve. This allowed for simple detection of changes in nifedipine induced lowering of MAP in the HL group after 0.1 mg kg^{-1} doses. HR was not used as a pharmacological measurement because an effect versus concentration relationship could not be identified. (Fig. 6-2.B)

Nifedipine is a low extracted drug in male Sprague-Dawley rats (169) eliminated by CYP450 3A in humans and an additional 2C subclass in the rat, (211). Previous work in our laboratory in P407-induced hyperlipidemic rats found a significant reduction (31%) in f_{up} of nifedipine (193). Since nifedipine is a low

extraction ratio drug in the rat the 31% decrease in f_{up} may explain the observed 40% decrease in CL_{sys} and subsequent increased AUC in the HL group (Table 6-1). These results are similar to those reports for cyclosporine A (103,212) and doxycycline (106) in hyperlipidemic states. Indeed, when AUC of nifedipine was corrected for the unbound fraction in plasma, no significant difference was observed between the NL and HL rat (Table 6-1). Nevertheless, a minor contribution of a decrease in Clu_{int} cannot be ruled out due to the increasing evidence suggestive of a decreased CYP450 content and activities in hyperlipidemic states (141). In addition, the possibility of a decreased hepatic uptake secondary to a down-regulation of LDL receptors in a hyperlipidemic condition exist (34).

Although we collected blood samples for the pharmacokinetic experiment for 120 min, the pharmacological effect of nifedipine was followed for only 30 min because in NL rats the effect did not last more than 30 min. In addition, maintenance doses of pentobarbital sodium that were required at 30 min decreased MAP. Although $AUC_{U(0\text{-}30min)}$ was significantly lower in the HL group as compared with the NL group, this however, did not result in a decrease in pharmacological effect as measured by $AUEC_{(0\text{-}30min)}$ and slope of the linear-effect curves (Table 6-2). Indeed, an increasing trend in effect, which amounted to a significantly higher effect at 30 min was observed (Fig. 6-6). This was accompanied by a significant prolongation of both $t_{1/2}$ of the concentration-time curve and pharmacological $t_{1/2}$ and a significant correlation between the two indices. A lack of a corresponding decrease or unaltered pharmacological effect

with a decrease or unaltered unbound nifedipine concentration, respectively, may suggest that MAP lowering is dependent on total rather than unbound nifedipine concentrations. This supports previous observations (98,118) that lipoproteins do not impair drug activity and may in fact facilitate drug uptake into tissue (213). The site of action of nifedipine is in vascular smooth muscle and, although the exact mechanism of binding is not determined, it has been suggested that nifedipine may reach its receptor by way of plasma lipids (144). This is plausible since lipoproteins interact with vascular smooth muscle, where LDL brings cholesterol to the smooth muscle and HDL removes it, evidenced by the role of LDL and HDL in atherosclerosis and reverse cholesterol transport (8), respectively. Perhaps nifedipine transport is augmented by plasma lipid transfer to the smooth muscle tissue.

Both HL and HLA groups tend to exhibit somewhat larger Vd_{ss} than NL and ATV groups (Table 6-1). These numerical differences resulted in statistical significance only between ATV and high cholesterol groups (HL and HLA). This may further support the notions of nifedipine distribution by plasma lipids. The NL goup showed the greatest variability in Vd_{ss}, which may explain its lack of significant differences with other groups.

Reduction of total plasma cholesterol levels using atorvastatin intervention (HLA group) increased CL_{sys} and decreased t½ of nifedipine to levels that were not significantly different than the NL group. Reduction of total plasma cholesterol in the HLA group significantly decreased pharmacological t½ of nifedipine in comparison to the HL group. However, pharmacological t½ remained significantly

higher in the HLA group as compared to the NL group. These findings are similar to a previous study in man using simvastatin in hyperlipidemic renal transplant recipients, where cyclosporine A trough concentrations decreased and f_{up} increased in simvastatin treated recipients in comparison to recipients (101). In addition, the significant positve correlations between drug concentrations and total plasma cholesterol levels observed for nifedipine are similar to previous findings for cyclosporine A (116) (189). Also, the observed significant inverse correlations between total plasma cholesterol and CL_{sys} of nifedipine are comparable to those reported for cyclosporine A (189).

Extrapolation of these results from rat to human is plausible given the similarities in alterations of cyclosporine A concentrations in plasma (103,189) and amphotericin B toxicities (116,214,214) in the hyperlipidemic rat and human.

Recently, nifedipine has been suggested to be associated with an increased mortality and cardiovascular events in subjects with established coronary heart disease (151). In addition, nifedipine induced reflex tachycardia, leading to a loss of blood pressure control, has been positively correlated, in part, to plasma concentrations. Perhaps future studies investigating the effects of hyperlipidemia on nifedipine PK and PD in human may help explain the large degree of inter and intra-variability of nifepine concentrations in plasma and the increased risk associated with nifedipine in coronary heart disease, a condition associated with hyperlipidemia.

6.3 Conclusions

Hyperlipidemia decreased CL_{sys} of nifedipine and increased $t_{1/2}$ without altering Vd_{ss}. Lowering of MAP was sustained and the pharmacological t½ was prolonged in the hyperlipidemic state, which corresponded with increases in total plasma nifedipine concentrations. Atorvastatin produced a 0 to 70% attenuation in P407-induced hyperlipidemia, which brought PK parameters within normal range. In addition, atorvastatin treatment normalized lowering of MAP, despite an incomplete normalization of pharmacological t½. The effect of nifedipine on MAP seems to correlate better with total rather than unbound plasma concentration. Thus high total plasma nifedipine concentrations in the hyperlipidemic state may alter its therapeutic outcome.

Table 6-1. Pharmacokinetic parameters of nifedipine (0.1 mg kg^{-1}) iv for the following groups: normolipidemia (NL, n=7), hyperlipidemia (HL, n=6), atorvastatin treated hyperlipidemia (HLA, n=8) or atorvastatin alone (ATV, n=6). Data is presented as arithmetic mean ± SD. Each group is compared to each other in the Statistical Comparisons column. Groups that are underlined together are not significantly different. n.d. Not determined.

	NL	ATV	HLA	HL	Statistical Comparisons
$AUC_{T(0-\infty)}$ ($\mu g\ ml^{-1}min$)	17.2 ± 2.6	17.9 ± 6.6	21.7 ± 5.8	28.5 ± 3.3	ATV NL HLA HL
$AUC_{U(0-\infty)}$ ($\mu g\ ml^{-1}min$)	0.6 ± 0.09	n.d.	n.d.	0.7 ± 0.08	NL HL
$AUC_{T(0-30min)}$ ($\mu g\ ml^{-1}min$)	8.3 ± 0.8	n.d.	n.d.	9.0 ± 1.2	NL HL
$AUC_{U(0-30min)}$ ($\mu g\ ml^{-1}min$)	0.3 ± 0.03	n.d.	n.d.	0.2 ± 0.02	NL HL
Cl_{sys} ($ml\ min^{-1}kg^{-1}$)	5.92 ± 0.93	6.14 ± 1.73	4.95 ± 1.54	3.55 ± 0.42	ATV NL HLA HL
$t_{½}$ (min)	34 ± 11	30 ± 12	46 ± 15	63 ± 14	ATV NL HLA HL
Vd_{ss} ($ml\ kg^{-1}$)	268 ± 44	227 ± 22	278 ± 35	309 ± 34	ATV NL HLA HL

Table 6-2. PD parameters of MAP lowering induced by nifedipine (0.1 mg kg^{-1}) iv for the following groups: normolipidemia (NL, n=7), hyperlipidemia (HL, n=6), atorvastatin treated hyperlipidemia (HLA, n=8) or atorvastatin alone (ATV, n=6). Data is presented as arithmetic mean ± SD. Each group is compared to each other in the Statistical Comparisons column. Groups that are underlined together are not significantly different.

	NL	ATV	HLA	HL	Statistical Comparisons
$AUEC_{(0\text{-}30)}$ (mm Hg*min)	407.8 ± 90.0	425.9 ± 175.3	372.0 ± 101.3	477.7 ± 93.1	ATV NL HLA HL
Max % decrease in MAP	37 ± 6	37 ± 2	32 ± 2	33 ± 5	ATV NL HLA HL
Pharmacological $t_{½}$ (min)	7 ± 2	7 ± 3	15 ± 8	28 ± 10	ATV NL HLA HL
Slope (m) of linear effect-concentration profile	0.097 ± 0.017	0.088 ± 0.026	0.084 ± 0.021	0.080 ± 0.036	ATV NL HLA HL

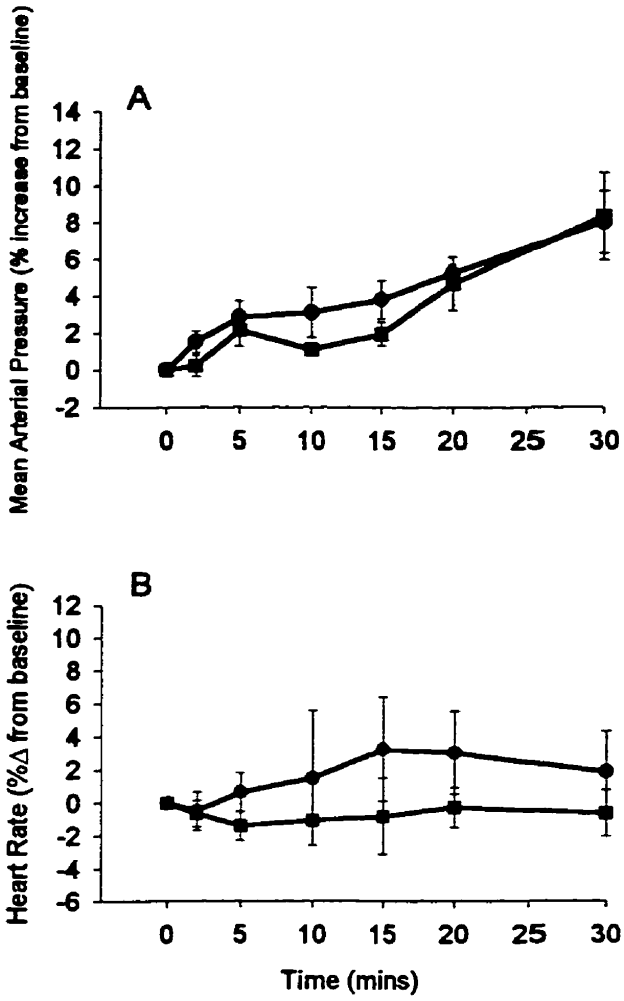

Fig. 6-1. Comparison of baseline (A) mean arterial pressure and (B) heart rate in normolipidemic (n=3) and hyperlipidemic (n=3) rats between time 0 - 30 min (Note: time 0 is start time for study, however it is 30 min after initial dose of pentobarbital sodium). Data is presented as mean ± SEM. No statistical differences were found.

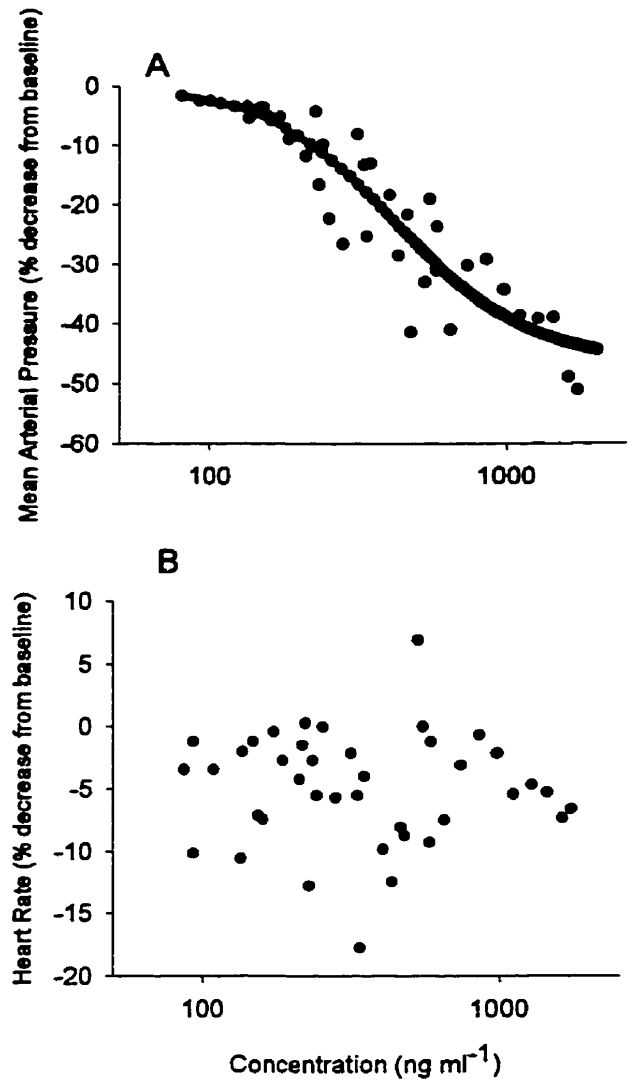

Fig. 6-2. Concentration-effect profile of percent decrease from baseline (A) mean arterial pressure and percent change from baseline (B) heart rate and nifedipine plasma concentration over the dose range of 0.05, 0.1 and 0.3 mg kg^{-1} in NL rats. The line in Fig 6-2 A. represents the best fit line for the sigmoidal-E_{max} model.

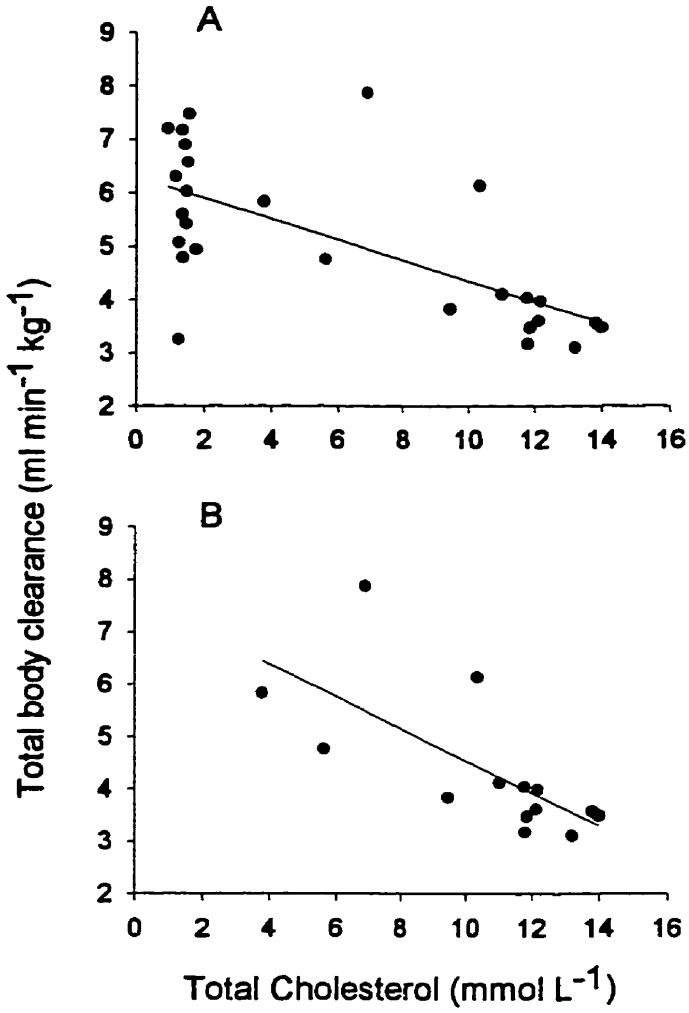

Fig. 6-3. (A). Correlation of nifedipine CL_{TB} (ml min^{-1} kg^{-1}) and total plasma cholesterol (mmol L^{-1}) between NL, ATV, HLA, and HL groups ($y = -0.19x + 6.29$, $r = 0.67$, $p = 0.004$). (B). Correlation of nifedipine CL_{TB} and total plasma cholesterol in the HL and HLA groups ($y = -0.31x + 7.61$, $r = 0.70$, $p = 0.028$).

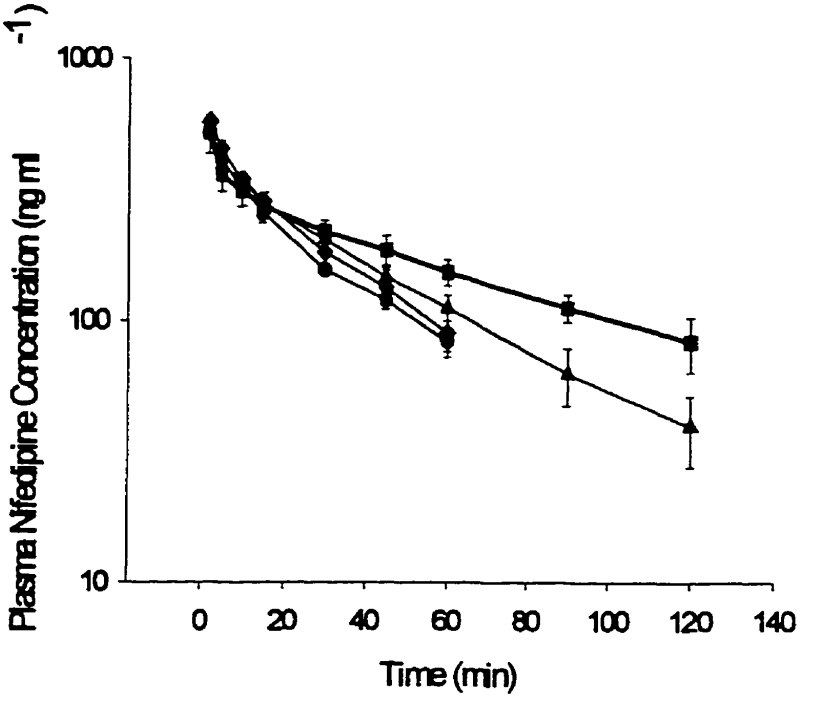

Fig. 6-4. Concentration-time profiles of plasma nifedipine (0.1 mg kg^{-1}, iv) in the NL (●, n=5), HL (■, n=6), HLA (▲, n=8) and A (◆, n=6) groups. Data is presented as arithmetic mean ± SEM. Plasma concentrations were elevated and sustained in the HL and HLA groups in comparison to the NL and A groups.

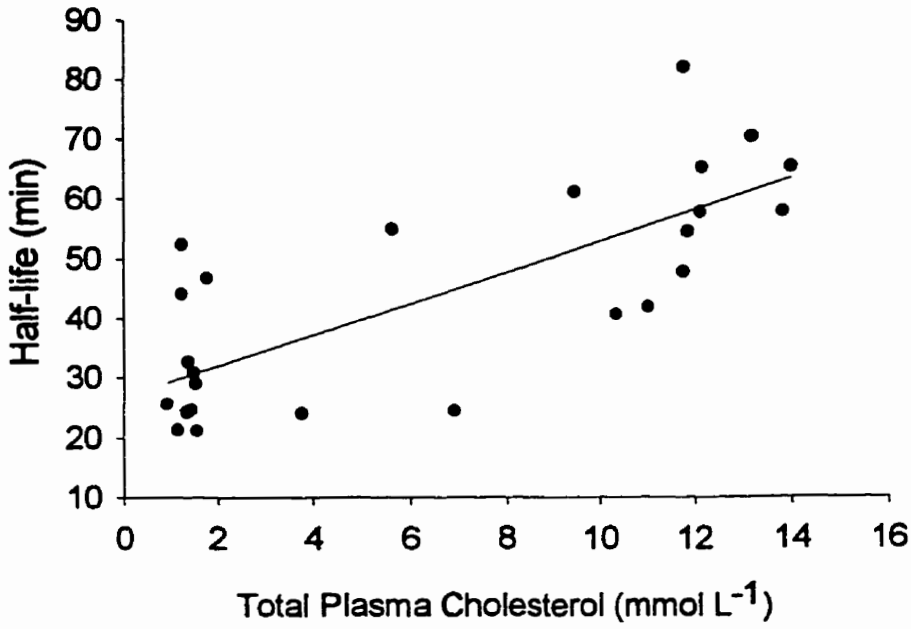

Fig. 6-5. Correlation of nifedipine t½ (min) and total plasma cholesterol (mmol L^{-1}) in NL, A, HLA, and HL groups ($y = 2.61x + 26.76$, $r = 0.76$, $p = 0.002$).

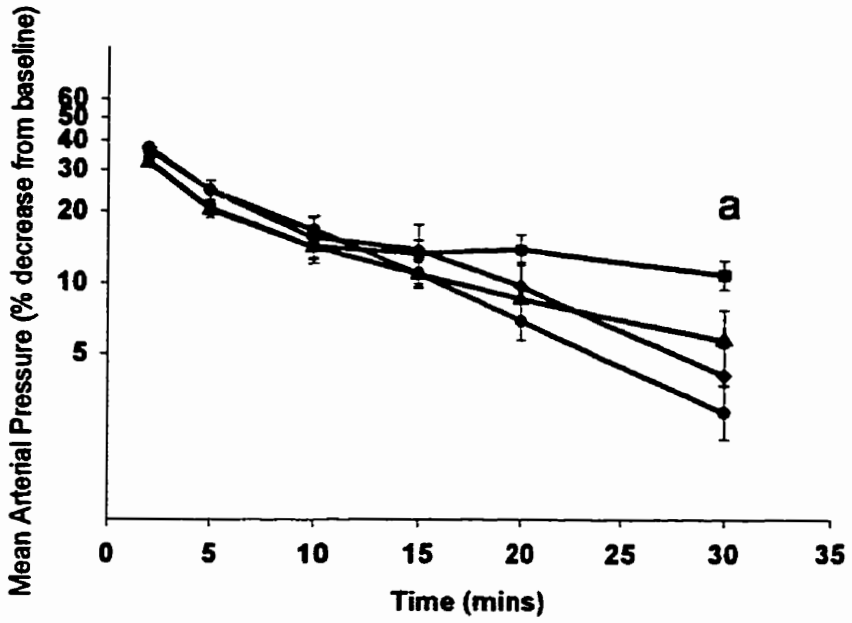

Fig. 6-6. Effect-time profiles of nifedipine (0.1 mg kg^{-1}, iv) in the NL (●, n=5), HL (■, n=6), HLA (▲, n=8) and A (◆, n=6) groups. Data is presented as arithmetic mean ± SEM. Each group is compared to the NL group and statistical differences ($p < 0.05$) are indicated by a. The percent decrease from baseline MAP was significantly higher in the HL group in comparison to the NL, HLA and A groups.

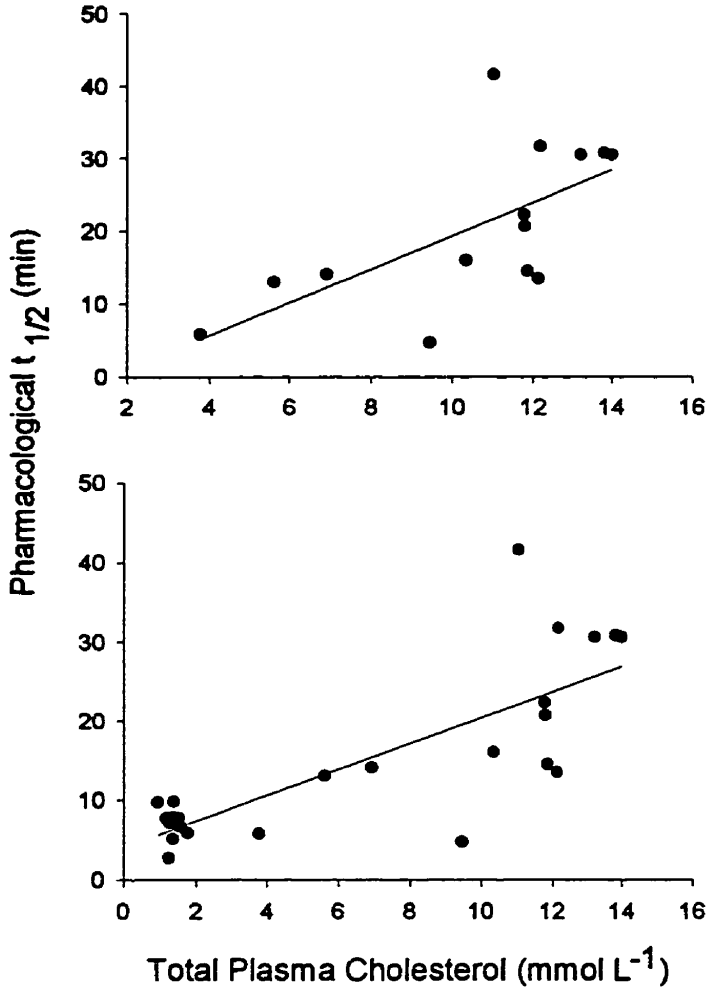

Fig. 6-7. (A). Correlation of nifedipine pharmacological t½ (min) and total plasma cholesterol (mmol L^{-1}) in NL, A, HLA, and HL groups ($y = 1.63x + 4.14$, $r = 0.80$, $p < 0.001$). (B). Correlation of nifedipine pharmacological t½ and total plasma cholesterol (mmol L^{-1}) in the HL and HLA groups ($y = 2.29x - 3.49$, $r = 0.64$, $p < 0.018$).

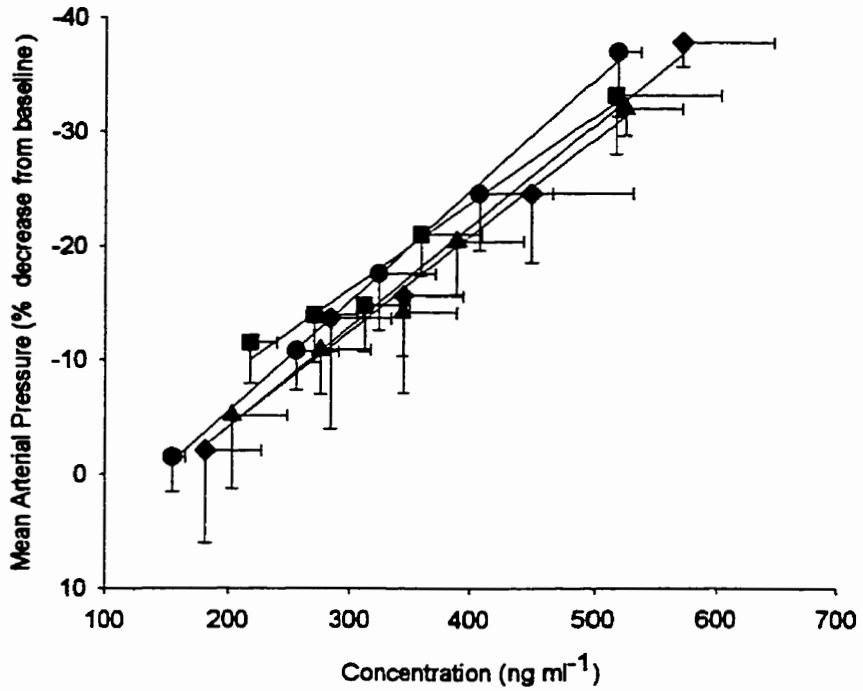

Fig. 6-8. Concentration-effect profiles of nifedipine (0.1 mg kg^{-1}, iv) in the NL (●, r^2 = 0.997, m = 0.097), HL (■, r^2 = 0.970, m = 0.075), HLA (▲, r^2 = 0.987, m = 0.084) and A (◆, r^2 = 0.990, m = 0.088 groups). Data is presented as arithmetic mean ± SEM. No statistical differences were found.

Chapter 7.0

General Discussions and Conclusions

7.1 History

Evidence suggesting that lipoproteins are important blood components for the binding and transport of basic drugs was provided in 1977 (215). In 1984 it was pointed out that little interest was shown for research into lipoprotein binding in comparison to the extensive research efforts evaluating albumin and α_1-acid glycoprotein binding (216). Perhaps the lack of interest was due to the presumption of a lack of clinical consequences of lipoprotein binding when considering the relatively low concentration of lipoproteins (about 10%) in plasma as compared with albumin (about 60%) (217). Currently, the significance of lipoproteins on total drug binding has been evaluated for many drugs, and can comprise between 5 - 95 percent of total drug binding (216). However, the consequences of variations in lipoprotein concentrations on the disposition and pharmacological responses of drugs have not been extensively studied (106). Nonetheless, the literature has evolved from case reports describing patterns of altered lipid levels in conjunction with altered drug concentrations and pharmacological effects (109,110,112) to studies describing complex details of drug distribution associated with lipoproteins and the corresponding drug transfer between lipoproteins and tissue (22). It is mainly thought that drug disposition is altered by hyperlipidemia due to an increase in plasma protein binding, which may still prove to be true. However, more recently, evidence suggests that hyperlipidemia causes a decrease in the content and activities of the CYP450 isozymes, the major enzymes responsible for biotransformation of many drugs (137,141,142).

Much of the increased interest in hyperlipidemic conditions has resulted from the severe (potentially life-threatening) conditions that hyperlipidemia is commonly associated with, including post-transplantation, cancer, viral infections (e.g. human immunodeficiency virus (HIV)), parasitic infections (e.g. malaria), and bacterial infections. Patients inflicted with these aforementioned diseases are commonly heavily medicated with a variety of drugs. Therefore, an understanding of the potential alterations of PK parameters, due to altered lipid levels and lipoprotein metabolism, is crucial.

7.2 PK and PD consequences of hyperlipidemia

Lipoproteins act as carriers for lipids, hydrophobic vitamins (213,218) and many drugs (99,219,220). It has been suggested that lipoproteins play a critical role in the extensive distribution and elimination of many drugs due to an extensive distribution of the lipoproteins into tissues (e.g. adipose, muscle, liver, kidney) (124,216). However, the magnitude of the role of lipoproteins on the distribution and elimination of drugs is most likely dependent on the capacity and binding affinity of drug to the lipoprotein, as well as the affinity of the drug to the relevant tissues (216).

Hepatic clearance (CL_H), AUC, Vd_{ss}, λ and $t_{1/2}$ (103,105-107,212) are altered for many drugs in hyperlipidemic conditions. Hyperlipidemic subjects showed decreases in doxycycline CL_{sys}, Vd_{ss} and λ and increases in AUC in comparison to normolipidemic subjects (106). Also, increases in AUC (107,212) and decreases in λ (212) with no changes in CL_{TB}/F and Vd_{ss}/F of both total and free phenytoin were found in similar comparisons. In addition, long term

administration of cyclosporine A in hyperlipidemic renal transplant recipients, resulted in increases in AUC and trough concentrations of cyclosporine A, which directly correlated with total, LDL and HDL cholesterol (113). In hyperlipidemic uremic patients a inverse linear relationship between cyclosporine A CL_{sys} and VLDL-triglyceride, VLDL-cholesterol and LDL-triglyceride concentrations resulted, however no correlation was found for LDL-cholesterol (189). Interestingly, these findings are similar to the observed inverse correlations found between nifedipine CL_{sys} and total plasma cholesterol disclosed in this thesis.

In many instances, disease-induced alterations of protein binding may alter pharmacological response(s) in a predictable manner, whereby a decrease in f_{up} causes either a decrease or unaltered C_u of drug or an increase in C_t of drug (93), which corresponds to a decrease, unaltered or increased pharmacological effect, respectively. It is evident that in some instances, hyperlipidemia affects pharmacological responses of drugs in a hither to, unpredictable manner, primarily due to the complexity of lipoprotein metabolism, distribution, and uptake at hepatic and extrahepatic sites (1,22).

7.3 PD of Amphotericin B in Hyperlipidemia

Studies investigating amphotericin B demonstrate many of the complexities regarding the mechanisms of hyperlipidemic-induced alterations in drug response as described above. Amphotericin B is an antifungal agent, commonly known to induce nephrotoxicity (221) and red blood cell hemolysis (118). In hyperlipidemic states antifungal activity towards *Candida albicans* remains (118), while red blood cell hemolysis decreases, indicating that

lipoproteins may serve as protective entities for red blood cells (118,125). The unaffected antifungal activity reported is likely a consequence of preferential binding of amphotericin B to fungal ergosterol rather than to mammalian cholesterol (222,223). Subsequently, administration of uncomplexed amphotericin B to hyperlipidemic rabbits caused less nephrotoxicity than amphotericin B complexed with LDL (125). Since LDL receptors are found on the kidney, increases in amphotericin B nephrotoxicity may be due to an increased delivery and internalization of LDL-associated amphotericin B within the kidney. An additional study reported that an increased affiliation of amphotericin B with LDL caused an increased incidence of nephrotoxicity in the obese hyperlipidemic Zucker rats (214). Further, in human subjects, an increase in amphotericin B-associated nephrotoxicity corresponded with elevated LDL-cholesterol levels (116). Conversely, amphotericin B appears not to cause nephrotoxicity when affiliated with HDL, because HDL does not interact with the kidney as LDL does, implying that an increase in amphotericin B-LDL binding corresponding to LDL-cholesterol levels may be responsible (116). It is known that amphotericin B preferentially binds to HDL as opposed to LDL (121,224), however, as LDL cholesterol increases, amphotericin B distribution in LDL also increases as shown in vitro (118). Therefore, it may be postulated that pharmacological responses of drugs that act on the liver or kidney (LDL receptor- prevalent tissues) may be altered due to changes associated with drug distribution among the lipoprotein classes, which is particularly relevant in hyperlipidemic conditions.

7.4 Mechanism(s) of Hyperlipidemic induced decreased CL_H

Although hyperlipidemia seems to alter drug concentrations and CL_H in a relatively consistent manner, the exact mechanism(s) have not been fully elucidated. The consequences of hyperlipidemia on CL_H are thought to be mediated by an increase in lipoprotein binding, resulting in a decreased f_{up} or fraction available (f_{av}) of drug for elimination. The commonly used hepatic elimination models (e.g. well-stirred, parallel tube, dispersion model) (225,226) assume that the f_{up} is the f_{av} for elimination. For example, with the "well-stirred" model,

$$CL_H = Q_H * \frac{CLu_{int} * f_{up}}{Q_H + (CLu_{int} * f_{up})}$$

Clearly, highly extracted drugs that are highly protein bound violate the assumption that f_{up} is the f_{av} for elimination. For example, propranolol is greater than 90 percent extracted and is highly protein bound (95 - 99%) (91,227), therefore $ER_H > f_{up}$. Therefore, the well-stirred model has been adapted (91) to account for this phenomenon as follows,

$$CL_H = Q_H * \frac{CLu_{int} * f_{av}}{Q_H + (CLu_{int} * f_{av})}$$

where f_{up} is replaced by f_{av} for elimination. LDL-receptor mediated uptake may provide, in part, a greater f_{av} for elimination, resulting in a $ER_H > f_{up}$. Many examples of drugs that are bound to lipoproteins and extracted to a greater

extent than their f_{up} exist (91,99,102,128,228). Notably, LDL receptor down-regulation commonly occurs in hyperlipidemic conditions (10,32,34), thereby decreasing the f_{av} for elimination, which may result in a decreased Cl_H.

7.5 PK and PD of Cyclosporine A in Hyperlipidemia

Cyclosporine A is a first-line immunosuppressant drug used to prevent graft rejection, post organ transplantation. With respect to hyperlipidemic-induced PK and PD alterations, it is a heavily investigated drug, because cholesterol and triglyceride levels are commonly elevated post organ transplantation. Cyclosporine A has a f_{up} ranging from 0.02 – 0.05 (91,229) and a ER_H of 0.2 - 0.5 (230). As well, the majority (> 80%) of total protein binding (> 95-99%) is mediated by lipoproteins and hepatic elimination is responsible for the majority of CL_{sys}. In two separate studies using hyperlipidemic Zucker rats, significant decreases in cyclosporine A CL_{sys} and Vd_{ss} (103) and increases in t½ and AUC (108) (103) occurred in comparison to equivalent studies using normal Sprague-Dawley rats. Although both authors concluded that the PK changes observed in the hyperlipidemic Zucker rats were due to an increase in protein binding, it has recently been shown that hepatic LDL receptors are down-regulated (34) and CYP450 is poorly expressed (141) in the hyperlipidemic Zucker rat. Therefore, alterations in the PK parameters of cyclosporine A in the hyperlipidemic Zucker rat may be a combination of a decrease in f_{up}, hepatic uptake, and CYP450 content and/or activity. In addition, since cyclosporine A elicits its effects in the reticular-endothelial system (RES), richly defined in the liver, down-regulation in LDL receptors, would be expected to cause a decrease in clinical efficacy. In

support of this 11 bone marrow transplant recipients with hypertriglyceridemia experienced graft rejection, despite the observed 11 – 30 fold increase in (109) cyclosporine A trough concentrations above the normal therapeutic range. In addition, the bone marrow transplant recipients experienced no nephrotoxicity. The lack of cyclosporine A effect and nephrotoxicity cannot be explained by the increased C_t of cyclosporine A, but could be caused by a down-regulation in LDL receptors. However, since C_u of cyclosporine A are unknown, conclusions as to the exact mechanism cannot be drawn. In addition, the largest study to date evaluating the effects of post-transplant hyperlipidemia on the f_{up} and clinical efficacy of cyclosporine A revealed a decrease in clinical efficacy with corresponding decreases in f_{up} (96). Over a 6 month period, f_{up} of cyclosporine A and total plasma cholesterol was determined for 1,848 plasma samples taken from 66 renal transplant recipients, and clinical response (i.e. graft rejection) was monitored and correlated to f_{up}, which varied 8 fold from 0.5 to 4.2 percent. Significant inverse correlations between f_{up} and HDL-cholesterol and apolipoprotein A were found. One week prior to rejection, a significant decrease in f_{up} of cyclosporine A was observed (96).

In contrast to the frequently observed decreases in CL_{sys} and Vd_{ss} in hyperlipidemic states, significant increases in CL_{sys} and Vd_{ss} of cyclosporine A in hypertriglyceridemic subjects caused by a high fat diet have been reported (114,115). It was conceded that the results were unexpected. Nonetheless, increases in cyclosporine A CL_{sys} were explained by suggesting that dietary fat acted as a carrier for the transport of cyclosporine A into the liver via chylomicron

remnants (114). Although not adequately investigated, this conclusion seems plausible since chylomicrons are taken up via chylomicron remnant receptors that are not down-regulated in response to high fat diets or hyperlipidemic conditions. This is in contrast to the commonly observed down-regulation of LDL receptors in response to high fat diets. The increase in Vd_{ss} was explained by suggesting that an increased diffusion of drug to adipocytes resulted from an increase in lipoprotein concentration after the high fat meal. Interestingly, in addition to the hypertriglyceridemia, a decrease in area under the total cholesterol-time profiles in subjects fed high fat diets occurred in comparison to subjects fed low fat diets (115). In this regard, it appears that a decrease in total plasma cholesterol may have been responsible for the increases in CL_{sys} and Vd_{ss}.

The lack of predictability of clinical efficacy of cyclosporine A using C_t and C_u in hyperlipidemic conditions has motivated researchers to develop a model accounting for total cholesterol and triglycerides for therapeutic drug monitoring of cyclosporine A (231). The model allows for estimation of f_{up} if total cholesterol and triglyceride levels are available, but since only 23 percent of the variation in f_{up} is accounted for by the model, the errors would be too large for use as a therapeutic drug monitoring tool.

7.5 Nifedipine

7.5.1 PK and PD Alterations in Hyperlipidemia

In the present context, hyperlipidemia did not alter presystemic elimination of nifedipine, as confirmed by the unchanged absolute bioavailability of

nifedipine. Since the gastrointestinal tract is responsible for the majority of nifedipine presystemic elimination in the rat, it may be suggested that the gastrointestinal CYP450 isozymes are not altered by enterohepatic recirculation of cholesterol. Clearly, P407-induced hyperlipidemia decreased CL_{sys} and f_{up}, and increased AUC and t½ of nifedipine similar to the effects of other hyperlipidemic models on many lipophilic entities (96). Since the ER_H of nifedipine is low in the rat (0.16 - 0.32) (169), it may be assumed that the CL_{sys} is dependent on f_{up} and CLu_{int} according to the most commonly used elimination models (e.g. well-stirred, parallel tube, dispersion model). In this regard, it appears that the 31 percent decrease in f_{up} of nifedipine explains the majority of the approximate 39 percent decrease in CL_{sys}. Nonetheless, since the ER_H (0.16 - 0.33) is greater than the f_{up} (0.01- 0.09) of nifedipine, a down-regulation in LDL receptors and a decrease in CYP450 content and activity, recently reported to occur in hyperlipidemic conditions (140-142) may also play a minor role in the decreased CL_{sys} and increased t½ observed. Furthermore, the increase in t½ suggests an increase in V_t or decrease in f_{ut}. These findings are in contrast to the decreased Vd_{ss} commonly observed with cyclosporine A. In this regard, the extent of drug distribution is determined by the competition for plasma protein versus tissue protein. Although cyclosporine A and nifedipine are highly distributed, the affinities of binding to lipoproteins and tissue proteins may be much different, which is a major determinant in tissue distribution. Historically, PK and PD studies in hyperlipidemic states have included only cyclosporine A and amphotericin B, therefore it is difficult to make generalized conclusions. Clearly,

the contribution of lipoproteins to the overall distribution and elimination of nifedipine needs to be determined prior to forming conclusions.

Although blood pressure lowering induced by nifedipine has previously been correlated to both C_u (172) and C_t (156,232,233) of nifedipine, it appears that C_t, rather than C_u of nifedipine corresponds with MAP lowering in the P407-induced hyperlipidemic rats. These results suggest that lipoproteins do not hinder the ability of nifedipine to distribute to its site of action, but may in fact aid in the transport of nifedipine to tissue receptor sites, which agrees with previous findings (98). For example, an increase in imipramine protein binding in hyperlipoproteinemic plasma *in vitro*, with no alterations in uptake into human fibroblasts (98), suggests that both bound and unbound drug was able to cross into the tissue.

Nifedipine elicits its action in vascular smooth muscle, however the exact mechanism of tissue uptake and binding to the calcium channels is undetermined. One hypothesis proposed that the delivery of 1,4 dihydropyridines to vascular smooth muscle is mediated by transfer with plasma lipids (144), which would explain the corresponding increases between C_t of nifedipine and the enhanced blood pressure lowering. This seems plausible given the role of LDL in atherosclerosis, whereby cholesterol is deposited into the intima layer of smooth muscle (8). In an opposite fashion, HDL transfers unesterified cholesterol from the intima to the liver by a process called reverse cholesterol transport (8). To date the exact mechanisms of lipid transfer are undetermined. Perhaps future studies, analyzing the interaction(s) of lipoproteins with vascular smooth muscle,

may increase the understanding of how hyperlipidemia may alter the clinical responses of nifedipine and other calcium channel blockers. Interestingly, it has been previously suggested that experimental work is required to elucidate the role of tissue uptake of dihydropyridine calcium antagonists in order to enable prediction of therapeutic outcomes in hyperlipidemic states (95).

7.5.2 Clinical Implications of Hyperlipidemia on Nifedipine

Many drugs that bind to lipoproteins have increased inter- and intrasubject PK variability (91,102,219,231) in contrast to those bound predominantly to albumin, since lipoprotein concentrations tend to fluctuate more than albumin, exaggerating the variability in f_{up} of lipophilic drugs (41,93,96,102,102,128). In humans, nifedipine concentrations exhibit a large degree of inter- and intrasubject variability (8 - 10 fold), exemplified by AUC and C_{max} values (178,234,235), which are associated with a high incidence of adverse events, because nifedipine toxicity is claimed to be dose-related (151). The adverse events of nifedipine are vasodilator-related. Therefore as blood pressure is lowered, common nifedipine-associated toxicities, such as headache, syncope, dizziness, flushing and tachycardia may be enhanced. Tachycardia is of utmost concern, because it may result in a loss of blood pressure control (156,177) and possibly an increased incidence of mortality (151). Interestingly, it is conceded that nifedipine immediate-release capsules are detrimental in subjects with coronary artery disease and post-myocardial infarction (154,158,236), conditions that are associated with hyperlipidemia. For this reason, nifedipine immediate-release formulations have been virtually replaced by sustained release products.

The sustained-release preparations of nifedipine reduce nifedipine plasma concentrations below levels expected to cause tachycardia (155,156,236,237), thereby considerably decreasing adverse events. However, a moderate incidence of tachycardia remains even with the sustained-release nifedipine formulations (32 versus 58 percent for immediate release) (238).

The prevalence of hyperlipidemia in subjects receiving nifedipine is inherently high, because nifedipine is commonly used in coronary heart disease or post-myocardial infarction. Studies assessing the effects of nifedipine in hyperlipidemic conditions in humans may lead to a better understanding of the potential cause(s) of the drugs apparent increased mortality risk in coronary artery disease, and help explain the ongoing controversy related to the safety of nifedipine. In addition, the high intra- and intersubject variability that leads to a moderately high incidence of adverse events may be explained by an elevation of total plasma cholesterol. Perhaps monitoring total plasma cholesterol levels and dose adjusting as required would clarify these issue(s), and, in turn, lead to a decrease in the risk(s) of nifedipine use.

7.6 HMG Co A Reductase Inhibitors

Cholesterol lowering using HMG CoA reductase inhibitors has proven to be of exceptional benefit in coronary heart disease (49-51). In addition, HMG Co A reductase inhibitors have been commonly used in heart transplant recipients to decrease the incidence of graft atherosclerosis (239,240). The potential normalization of drug PK parameters in hyperlipidemic conditions by lowering total plasma cholesterol and triglyceride levels was recently advocated (101).

Treatment of subjects with simvastatin before and after heart transplantation produced a significant decrease in total and LDL cholesterol of approximately 13 and 23 percent, respectively (101). In turn, the f_{up} of cyclosporine A increased from 1.4 ± 0.1 to 1.82 ± 0.22 and mean trough concentrations decreased from 349 to 242 ug L^{-1}, respectively. These findings are similar to the effects observed on nifedipine PK parameters following atorvastatin administration in the P407-induced hyperlipidemic rat. Specifically, cholesterol lowering normalized the AUC and CL_{sys}, and decreased t½ of nifedipine. In addition, a sustained lowering of MAP in the hyperlipidemic state was partially, although not completely normalized after treatment with atorvastatin, as evidenced by a significant decrease in pharmacological t½. To date, these two studies are among the first to evaluate the effects of cholesterol lowering on the PK parameters of drugs in the hyperlipidemic state. Importantly, the observed normalization of the PK parameters after cholesterol reduction are encouraging, indicating that HMG CoA reductase inhibitors may normalize PK and PD alterations induced by hyperlipidemia for many drugs. Therefore, future human studies, elucidating the roles of HMG CoA reductase inhibitors on the PK and PD of drugs in hyperlipidemic conditions, should be required.

Many disease conditions create physiological changes that alter the clinical efficacy of drugs, which may not be overcome by simple co-administration of therapeutic entities. The potential of regulating cholesterol and triglyceride levels to produce optimal clinical efficacy of these drugs seems to be a simple solution for a larger problem.

7.7 Calcium Channel Blockers

Previous studies have shown that lipoproteins are major determinants in plasma protein binding for many calcium antagonists other than nifedipine including; diltiazem (41), nicardipine (102), darodipine (130), isradipine and amlodipine (95). To date, that effects of hyperlipidemia on the PK and PD of these calcium channel blockers have not been determined, with the exception of nifedipine. The consequences of hyperlipidemia on nifedipine PK and PD cannot be extrapolated to the other calcium channel blockers, because the mechanisms of lipoprotein binding may be different, due to differing lipophilicity, physiochemical properties, and disposition. Generally, calcium channel blockers are administered to patients with secondary hyperlipidemia, and like nifedipine, hyperlipidemia may play a role regarding inter- and intrasubject variability in the PK and PD parameters of calcium channel blockers.

7.8 Obstacles in Lipid Research

Hyperlipidemic models have been described in many animal species. The cholesterol fed rabbit and the Watanabe rabbit, a gene knock-out model of heterozygous familial hyperlipidemia (241,242), have been the most popular models utilized in lipid research to date. Unfortunately, the use of rabbits in PK and PD studies are inconvenient and have a considerable expense attached. Alternatively, the ease of handling and low costs of the rat model has resulted in its common use for experimental research. Clearly, the anatomical structures and physiological processes of the rat have been extensively documented (181).

Furthermore, the disposition of many xenobiotics has been evaluated and many models of disease are established and validated in the rat.

The potential extrapolation of findings to human is an important factor in experimental research. In this regard, it has been suggested that the rat model has limitations in lipid research, particularly due to the differences in normal basal total plasma cholesterol levels and lipoprotein distribution between the human and rat. However, previously, the majority of lipid research has focused on the study of the causes and processes of coronary heart disease and the discovery of therapeutic entities to halter these processes. Considering that LDL is the major contributor to coronary heart disease (43,184,241) and it makes up only 20 – 30 percent of total lipoprotein concentrations in the rat compared to 60 – 80 percent in the human, it appears the reported concerns have merit. On the contrary, arguments against the use of the rat model for investigating the causal effects of hyperlipidemia on the disposition of drugs are less well founded. In fact, similar PK and PD consequences with cyclosporine A and amphotericin B in rat (103,108,214) and human (113,116,189,243) have been reported. The majority of studies evaluating the consequences of hyperlipidemia on the PK and PD of drugs have found corresponding decreases in f_{up} and CL_{sys}. A concern remains however, regarding the drugs affinity and selectivity for the individual lipoprotein sub-classes, since the resulting transport and uptake of lipoproteins by tissue may preclude the use of the rat model for extrapolation to the human condition. For example, HDL and LDL both interact with peripheral tissues, but differences in tissue selectivity have been noted. Nonetheless, the significance of these

differences between rat and human will not be determined until the mechanism(s) by which hyperlipidemia alters the PK and PD parameters of drugs is fully elucidated.

7.9 P407-Induced Hyperlipidemic Rat Model

The P407-induced hyperlipidemic rat model was introduced in 1995, therefore it is a relatively new model. The idea of surfactant-induced hyperlipidemia has been know for many years, beginning with Triton WR 1339 (73), which unfortuately caused cellular toxicities (74). Although P407 e has been regarded as non-toxic (76),(75,78,83,87,88,182) pathological and histological assessment of short- and long-term administrations of P407 were studied in this research project. The pathological assessments confirmed the findings of previous reports (87), demonstrating that P407 does not disrupt cellular membranes. P407 appears, for the most part, to be inert, other than inducting hyperlipidemia and lipid infiltration into various tissues.

7.10 Conclusions

1. Single dose administration of 1 g kg^{-1} P407, ip, and every 96 h for 4 and 6 wk induces hyperlipidemia, but otherwise, rats were healthy. One rat, at 6 wk had lipid infiltration in intimal cells of the aorta, of the foam cell type, which may be indicative of early stages of atherosclerosis. Nine weeks of the similar dosage regime produced lipid infiltration into various tissues. Therefore, since no apparent gross morphological and pathohistological alterations were observed, the P407-induced hyperlipidemic rat model may be used to

evaluate the effects of hyperlipidemia on PK and PD parameters of drugs after acute and chronic dosing (up to 6 wk).

2. P407-induced hyperlipidemia decreased nifedipine CL_{TB} and CL_{TB}/F, and increases AUC and t½ with no significant alterations in Vd_{ss}. The increases in total plasma nifedipine concentrations in hyperlipidemic rats produce a sustained lowering of MAP, evidenced by the increased pharmacological t½. In addition, significant positive correlations between total plasma cholesterol and AUC, t½ and pharmacological t½, as well as inverse correlations with Cl_{TB} were found.
3. P407-induced hyperlipidemia decreases the f_{up} of nifedipine in plasma, which appears to be the major contributor to the decreased CL_{TB}. However a decrease in hepatic uptake, CYP450 content and activity and p-glycoprotein activity cannot be ruled out.
4. HMG CoA reductase inhibitors, lovastatin and atorvastatin, decrease P407-induced hyperlipidemia similarly. However the efficacy is dependent on the particular dosing regime. In particular, HMG CoA reductase inhibitors must be given 24 h prior to administration of P407 and dosed only once daily, as opposed to twice daily.
5. Inhibition of P407-induced hyperlipidemia by atorvastatin results in significant normalizations of nifedipine PK and PD parameters of nifedipine, however not complete.

6. In treating hyperlipidemic patients with nifedipine, attention should be paid to the possibility of altered PD outcomes due to a potential increase in protein binding and/or a decrease in metabolism.

Reference List

1. C. J. Fielding and P. E. Fielding. Metabolism of cholesterol and lipoproteins. In D. E. Vance and J. E. Vance (eds.), *Biochemistry of lipids and membranes*, The Benjamin/Cummings Publishing Company, Inc., Menlo Park, 1985, pp. 404-474.

2. P. Yeagle. Cholesterol and the cell membrane. *Biochim.Biophys.Acta* **822:** 267-287 (1985).

3. B. V. Khan, TV. Fungwe, HG. Wilcox, and M. Heimberg. Cholesterol is required for the secretion of the very-low-density lipoprotein: in vivo studies. *Biochim.Biophys.Acta* **1044:** 304 (1990).

4. B. Khan, H. G. Wilcox, and M. Heimberg. Cholesterol is required for secretion of very-low-density lipoprotein by rat liver. *Biochem.J.* **259:** 807-816 (1989).

5. A. W. Alberts. Discovery, Biochemistry and Biology of Lovastatin. *Am.J.Cardiol.* **62:** 10J-15J (1988).

6. K. Bloch. Cholesterol:Evolution of structure and function. In D. E. Vance and J. E. Vance (eds.), *Biochemistry of lipids and membranes*, The Benjamin/Cummings Publishing Company, Inc., Menlo Park, 1985, pp. 1-24.

7. V. W. Rodwell, J. L. Nordstrom, and J. J. Mitschelen. Regulation of HMG-CoA reductase. *Adv.Lip.Res.* **14:** 1-74 (1976).

8. P. E. Fielding and C. J. Fielding. Dynamics of lipoprotein transport in the human circulatory system. In D. E. Vance and J. E. Vance (eds.), *Biochemistry of Lipids, Lipoproteins, and Membranes.*, Elsevier, Paris, 1996, pp. 495-541.

9. C. Edelstein, F. Kezdy, A. M. Scanu, and B. W. Shen. Apolipoproteins and the structural organization of plasma lipoproteins:Human plasma high density lipoprotein-3. *J.Lip.Res.* **20:** 143 (1979).

10. M. S. Brown and J. L. Goldstein. A receptor-mediated pathway for cholesterol homeostasis. *Science* **23:** 34-47 (1986).

11. R. J. Havel and J. P. Kane. Introduction: Structure and metabolism of plasma lipoproteins. In C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (eds.), *The metabolic and molecular basis of inherited disease.Vol. II.*, McGraw-Hill, Inc., New York, 1995, pp. 1841-1851.

12. E. J. Schaefer and R. Levy. Pathogenesis and management of lipoprotein disorders. *N.Engl.J.Med.* **312:** 1300-1310 (1985).

13. H. N. Ginsberg. Lipoprotein metabolism and its relationship to atherosclerosis. *Med.Clin.North Am.* **78:** 1-20 (1994).

14. R. Poledne. Cholesterol metabolism in prague hereditary hypercholesterolemic rat. In M. J. Halpern (ed.), *Lipid metabolism and its pathology. Proceedings of the IVth international colloquium on lipid*

metabolism and its pathology., Excerpta medica, Amsterdam, 1986, pp. 185-189.

15. J. A. Porter, B. L. Carter, T. P. Johnston, and W. K. Palmer. Effects of pravastatin on plasma lipid concentrations in poloxamer 407-induced hyperlipidemic rats. *Pharmacotherapy* **15:** 92-98 (1995).

16. R. A. Davis and J. E. Vance. Structure, assembly and secretion of lipoproteins. In D. E. Vance and Vance.J. (eds.), *Biochemistry of Lipids, Lipoproteins and Membranes.*, Elsevier, Edmonton, 1996, pp. 473-493.

17. Y. Oschry and S. Eisenberg. Rat plasma lipoproteins: re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. *J.Lip.Res.* **23:** 1099-1106 (1982).

18. D. K. Spady, S. D. Turley, and J. M. Dietschy. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J.Lipid Res.* **26:** 465-472 (1985).

19. R. C. Kowal, J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc.Natl.Acad.Sci.* **86:** 5810 (1989).

20. M. S. Brown and J. L. Goldstein. Lipoprotein receptors in the liver. Control signals for plasma cholesterol traffic. *J.Clin.Invest.* **72:** 743-747 (1983).

21. H. Jingami and T. Yamamotot. The VLDL receptor:wayward brother of the LDL receptor. *Curr.Opin.Lipidol.* **6:** 104-108 (1995).

22. K. M. Wasan and S. M. Cassidy. Role of Plasma Lipoproteins in Modifying the Biological Activity of Hydrophobic Drugs. *J.Pharm.Sci.* **87:** 411-424 (1998).

23. J. Shepherd. Lipoprotein Metabolism. *Drugs* **47:** 1-10 (1994).

24. K. Rodondi. Hyperlipidemia. *Therapeutics*, 1997, pp. 387-403.

25. P. J. Barter and J. I. Lally. The activity of an esterified cholesterol transfering factor in human and rat serum. *Biochim.Biophys.Acta* **531:** 233-236 (1978).

26. E. Bravo, A. Cantafora, and L. Argiolas. Hepatic uptake and metabolism of free cholesterol from different lipoprotein classes. An in vivo study in the rat. *Biochim.Biophys.Acta, Ser Lipids Lipid Metab* **100.:** 315-320 (1989).

27. C. J. Packard. Plasma lipid and lipoprotein metabolism in the 1990s - what we know and what we need to know. In D. J. Betteridge (ed.), *Lipids: Current Perspectives*, Mosby, London, 1996, pp. 1-20.

28. V. Guyarddangremont, C. Desrumaux, P. Gambert, C. Lallemant, and L. Lagrost. Phospholipid and cholesteryl ester transfer activities in plasma from 14 vertebrate species - relation to atherogenesis susceptibility. *Comparative Biochemistry & Physiology - B: Comparative Biochemistry.* **120:** 517-525 (1998).

29. M. Rudling. *J.Lipid Res.* **33:** 493-501 (1992).

30. P. D. Roach, S. Balasubramaniam, F. Hirata, M. Abbey, A. Szanto, L. A. Simons, and P. J. Nestel. The low-density lipoprotein receptor and cholesterol synthesis are affected differently by dietary choleterol in the rat. *Biochim.Biophys.Acta* **1170:** 165-172 (1993).

31. A. S. Truswell and J. I. Mann. Epidemiology of serum lipids in Southern Africa. *Atherosclerosis* **16:** 15-19 (1972).

32. R. Talbert. Hyperlipidemia. *DePiro Textbook of Therapeutics*, Appleton and Lange, Connecticut, 1997, pp. 357-386.

33. D. K. Spady and J. A. Cuthbert. The regulation of hepatic sterol metabolism in the rat. *J.Biol.Chem.* **267:** 5584-5591 (1992).

34. W. Liao, B. Angelin, and M. Rudling. Lipoprotein metabolism in the fat Zucker rat: reduced basal expression but normal regulation of hepatic low density lipoprotein receptors. *Endocrinology* **138:** 3282 (1997).

35. M. Rudling, G. Norstedt, H. Olivecrona, E. Reihner, J. Gustafsson, and B. Angelin. Importance of growth hormone for the induction of hepatic low density lipoprotein receptors. *Proc.Natl.Acad.Sci.* **89:** 6983-6987 (1992).

36. M. Rudling and B. Angelin. Loss of resistance to dietary cholesterol in the rat after hypophysetomy: Importance of the presence of growth hormone for

hepatic low density lipoprotein-receptor expression. *Proc.Natl.Acad.Sci.* **90:** 8851-8855 (1993).

37. G. S. Tannenbaum and J. B. Martin. *Endocrinology* **98:** 562-570 (1976).

38. K. R. Feingold, R. M. Krauss, M. Pang, W. Doerrler, P. Jensen, and C. Grunfeld. The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low-density lipoprotein subclass pattern. *J.Clin.Endocrinol.Metab.* **76:** 1423-1431 (1993).

39. B. A. Beutler and A. Cerami. Recombinant interleukin-1 suppresses lipoprotein lipase activity in 3T3-L1 cells. *J.Immunol.* **135:** 3969-3971 (1985).

40. J. S. Patton, H. M. Shepard, and H. Wilking. Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. *Proc.Natl.Acad.Sci.* **83:** 8313-8317 (1986).

41. T. C. Kwong, J. D. Sparks, and C. E. Sparks. Lipoprotein and protein binding of the calcium channel blocker diltiazem. *Proc.Soc.Exp.Biol.Med.* **178:** 313-316 (1985).

42. J. D. Pirsch, A. M. D'Alessandro, and H. W. Sollinger. Hyperlipidemia and transplantation:etiologic factors and therapy. *J.Am.Soc.Nephrol.* **2:** 1992).

43. W. B. Kannel, W. P. Castelli, and T. Gordon. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann.Intern.Med.* **90:** 85-91 (1979).

44. L. Simons. Interrelations of lipids and lipoproteins with coronary artery disease mortality in 19 countries. *Am.J.Cardiol.* **57:** 5G-10G (1986).

45. V. Manninen, L. Tenkanen, and P. Koskenen. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation* **85:** 3745 (1992).

46. W. P. Castelli, J. T. Doyle, and T. Gordon. HDL-cholesterol and other lipids in coronary heart disease: The Cooperative Lipoprotein Phenotyping Study. *Circulation* **55:** 767-772 (1977).

47. P. Deegan and J. Feely. Making sensible drug choices - horses for courses. In D. J. Betteridge (ed.), *Lipids:Current Perspectives*, Mosby, London, 1996, pp. 227-240.

48. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian survival study (4S). *Lancet* **344:** 1383-1389 (1994).

49. J. R. I. Crouse, R. P. Byington, and M. G. Bond. Pravastatin, lipids, and atherosclerosis in the carotid arteries.PLACII. *Am.J.Cardiol.* **75:** 445-459 (1995).

50. J. Shepherd, S. M. Cobbe, and I. Ford. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N.Engl.J.Med.* **333:** 1301-1307 (1995).

51. G. Brown, J. J. Albers, and L. D. Fisher. Regression of coronary artery disease as a result of intensive lipid-lowering in men with high levels of apo-B. *N.Engl.J.Med.* **323:** 1289-1298 (1990).

52. Parke-Davis and Warner-Lambert Company. Product Monograph - Lipitor - Lipid Metabolism Regulator. 1997. Scarborough, Ontario.

53. CR. Sirtori. Pharmacology and mechanism of action of the new HMG-CoA reductase inhibitors. *Pharmacol.Res.* **22:** 555-563 (1990).

54. S. M. Grundy. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. *N.Engl.J.Med.* **319:** 24-33 (1988).

55. P. H. Chong and J. D. Seeger. Atorvastatin Calcium: An addition to HMG-CoA Reductase Inhibitors. *Pharmacotherapy* **17:** 1157-1177 (1997).

56. H. Lennernas and G. Fager. Pharmacodynamics and Pharmacokinetics of the HMG-CoA Reductase Inhibitors. *Clin.Pharm.* **32:** 1997).

57. P.Alaupovic, T.Heinonen, L.Shurzinski, and D.M.Black. Effect of a new HMG-CoA reductase inhibitor, atorvastatin, on lipids, apolipoproteins and lipoprotein particles in patients with elevated serum cholesterol and triglyceride levels. *Atherosclerosis* **113:** 123-133 (1997).

58. Atorvastatin Study Group. Comparison of one-year efficacy and safety of atorvastatin versus lovastatin in primary hypercholesterolemia. *Am.J.Cardiol.* **79:** 1475-1481 (1997).

59. Atorvastatin - A New Lipid-Lowering Drug. *The Medical Letter* **39:** 29-32 (1997).

60. Atorvastatin Calcium. *Drugs of the Future* **22:** 959-968 (1997).

61. R.L .Susick and T.Woolfe. Application of toxicokinetic principles in the development of antiatherosclerotic compounds. In P.G.Welling and F.A.De La Iglesia (eds.), *Drug Toxicokinetics*, Marcel Dekker, New York, 1993, pp. 305-324.

62. T. M. Bocan, E. Ferguson, and W. McNally. Hepatic and nonhepatic sterol synthesis and tissue distribution following administration of a liver selective HMG-CoA reductase inhibitor, CI-981: comparison with selected HMG-CoA reductase inhibitors. *Biochim.Biophys.Acta* **1123:** 133-144 (1992).

63. Parke-Davis Pharmaceuticals. Atorvastatin (Lipitor) oral package insert. *Arbor, A.MI* 1996).

64. H. Y. Pan, A. R. DeVault, D. Wang-Iverson, E. Ivashkiv, B. N. Swanson, and A. A. Sugerman. Comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. *J.Clin.Pharmacol.* **30:** 1128-1135 (1990).

65. P.J.Pentikainen, M.Saraheimo, J.I.Schwartz, R.D.Amin, M.S.Schwartz, F.Bunner-Ferber, and J.D.Rogers. Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. *J.Clin.Pharmacol.* **32:** 140 (1992).

66. S. Fantappie, M. Crestani, E. Bosisio, G. Galli, F. Maggi, A. Corsini, and A. Catapano. Plasma lipoproteins and cholesterol metabolism in spontaneously hyperlipemic rats. *Atherosclerosis* **76:** 171 (1989).

67. J. Russell, D. Koeslag, R. Amy, and P. Dolphin. Plasma lipid secretion and clearance in hyperlipidemic JCR:LA-corpulent rats. *Arteriosclerosis* **9:** 869-876 (1989).

68. L. St.John and F. Bell. Arterial lipid biochemistry in the spontaneously hyperlipidemic Zucker rat and its similarity to early atherogenesis. *Atherosclerosis* **86:** 139-144 (1991).

69. R. J. Martin, P. J. Wangsness, and J. H. Gahagan. Diurnal changes in serum metabolites and hormones in lean and obese Zucker rats. *Horm.Metab.Res.* **10:** 187-192 (1978).

70. K. Imaizumi, A. Nagatomi, M. Sato, A. Tominaga, and M. Sugano. Cholesterol metabolism in ExHC (exogenous hypercholesterolemic) rats. *Biochim.Biophys.Acta* **1123:** 101-109 (1992).

71. K. R. Muller, J. R. Li, D. M. Dinh, and M. T. R. Subbiah. The characteristics and metabolism of a genetically hypercholesterolemic strain of rats. *Biochim.Biophys.Acta* **574:** 334 (1979).

72. T. Ishikawa and N. Fidge. Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats. *J.Lip.Res.* **20:** 254-264 (1979).

73. S. Otway and D. S. Robinson. The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J.Physiol.* **190:** 321-332 (1967).

74. M. Okazaki, M. Suzuki, and K. Oguchi. Changes in coagulative and fibrinolytic activities in Triton WR-1339 induced hyperlipidemia in rats. *Jpn.J.Pharmacol.* **52:** 353-361 (1990).

75. I. R. Schmolka. Poloxamer in the Pharmaceutical Industry. In P. J. Tarcha (ed.), *Polymers for controlled drug delivery.*, 19 A.D., pp. 189-214.

76. BASF Corporation. Pluronic polyols: Toxicity and irritation data. *Publication No.3012* **767:** 1-32 (1978).

77. P. Wang and T. P. Johnston. The kinetics of the sol-to-gel transition for poloxamer polyols. *J.Appl.Polym.Sci.* **43:** 283-292 (1991).

78. E.A.Pec, Z.G.M.Wout, and T.P.Johnston. Biological activity of urease formulated in poloxamer 407 after intraperitoneal injection in the rat. *J.Parenter.Sci.Technol.* **81:** 626-630 (1992).

79. T. P. Johnston, M. A. Punjabi, and C. J. Froelich. Sustained delivery of interleukin-2 from a poloxamer 407 gel matrix following intraperitoneal injection in mice. *Pharm.Res.* **9:** 421-430 (1992).

80. T. P. Johnston and W. K. Palmer. Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. *Biochem.Pharmacol.* **46:** 1037-1042 (1993).

81. Z. G. M. Wout, E. A. Pec, J. A. Maggiore, R. H. Williams, P. Palicharla, and T. P. Johnston. Poloxamer 407-mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. *J.Parenter.Sci.Technol.* **46:** 192-200 (1992).

82. T. P. Johnston and W. K. Palmer. Effect of poloxamer 407 on the activity of microsomal 3-hydroxy-3-methylglutaryl CoA reductase in rats. *J.Cardiovasc.Pharmacol.* **29:** 580-585 (1997).

83. V. J. Nash, T. P. Johnston, and W. K. Palmer. Effects of nicotinic acid on poloxamer 407-induced hyperlipidemia. *Pharmacotherapy* **16:** 10-15 (1996).

84. C. Li, W. K. Palmer, and T. P. Johnston. Disposition of poloxamer 407 in rats following a single intraperitoneal injection assessed using a simplified colorimetric assay. *J.Pharm.Biomed.Anal.* **14:** 659-665 (1996).

85. I. J. Torres, C. L. Litterst, and A. M. Guarino. Transport of model compounds across the peritoneal membrane in the rat. *Pharmacology* **17:** 330-340 (1978).

86. D. Attwood and A. T. Florence. Surfactant Systems: their chemisty, pharmacy, and biology. Chapman and Hall, New York, 1983, pp. 619.

87. T. P. Johnston and S. C. Miller. Toxicological evaluation of poloxamer vehicles for intramuscular use. *J.Parent.Sci.Tech.* **39:** 83-88 (1985).

88. T. P. Atkinson, J. O. Bullock, T. F. Smith, R. E. Mullins, and R. L. Hunter. Ion transport mediated by copolymers composed of polyoxyethylene and polyoxypropylene. *Am.J.Physiol.* **254:** C26 (1988).

89. T. P. Johnston, H. Beris, Z. G. M. Wout, and J. L. Kennedy. Effects on splenic, hepatic, hematological, and growth parameters following high-dose poloxamer 407 administration to rats. *Int.J.Pharm.* **100:** 279-284 (1993).

90. M. Rowland. Plasma protein binding and therapeutic monitoring. *Ther.Drug Monitor.* **2:** 29-37 (1980).

91. J. MacKichan. Influence of Protein Binding and Use of Unbound (Free) Drug Concentrations. In W. Evans, J. Schentag, and W. Jusko (eds.), *Applied Pharmacokinetics - Principles of Therapeutic Drug Monitoring*, Applied Therapeutics, Inc., Vancouver, 1992, pp. 5-1-5-48.

92. W.J.Jusko and M.Gretch. Plasma and tissue protein binding of drugs in pharmacokinetics. *Drug Metab.Rev.* **14:** 427 (1983).

93. K. M. Piafsky. Disease-induced changes in the plasma binding of basic drugs. *Clin Pharmacokinet.* **5:** 246-262 (1980).

94. F. Herve, S. Urien, E. Albengres, J.-C. Duche, and J.-P. Tillement. Drug Binding in Plasma. A summary of recent trends in the study of drug and hormone binding. *Clin Pharmacokinet.* **26:** 44-58 (1994).

95. J. Oravcova, D. Sojkova, N. Fetkovska, and T. Trnovec. Factors influencing isradipine and amlodipine binding to human plasma lipoproteins. *Blood Press.* **1:** 61-64 (1994).

96. A. Lindholm and S. Henricsson. Intra- and interindividual variability in the free fraction of cyclosporine in plasma in recipients of renal transplants. *Ther.Drug Monit.* **11:** 623-630 (1989).

97. A. M. Gardier, D. Mathe, X. Guedeney, J. Barre, C. Benvenutti, N. Navarro, L. Vernilley, D. Loisance, J.-P. Cachera, B. Jacotot, and J. P. Tillement. Effects of plasma lipid levels on blood distribution and pharmacokinetics of cyclosporine A. *Ther.Drug Monit.* **15:** 274-280 (1993).

98. A. Danon and Z. Chen. Binding of imipramine to plasma proteins: Effect of hyperlipoproteinemia. *Clin.Pharm.Ther.* **25:** 316-321 (1998).

99. E. Pike, B. Skuterud, P. Kierulf, and P. K. Lunde. Significance of lipoproteins in serum binding variations of amitriptyline, nortriptyline and quinidine. *Clin.Pharm.Ther.* **32:** 599-606 (1982).

100. S. Bower. Plasma protein binding of fentanyl: the effect of hyperlipoproteinaemia and chronic renal failure. *J.Pharm.Pharmacol.* 102-106 (1982).

101. F. Akhlaghi, A. McLacklan, A. Keogh, and K. Brown. Effect of simvastatin on cyclosporine unbound fraction and apparent blood clearance in heart transplant recipients. *Br.J.Clin.Pharmacol.* **44:** 537-542 (1997).

102. S. Urien, E. Albengres, A. Comte, J. R. Kiechel, and J. P. Tillement. Plasma protein binding and erythrocyte partitioning of nicardipine in vitro. *J.Cardiovasc.Pharmacol.* **7:** 891-898 (1985).

103. L. J. Brunner, K. Vadiei, and D. R. Luke. Cyclosporine disposition in the hyperlipidemic rat model. *Res.Commun.Chem.Pathol.Pharmacol.* **59:** 339-348 (1988).

104. G. M. Kuster, H. Drexel, J. A. Bleisch, K. Rentsch, P. Pei, U. Binswanger, and F. W. Amann. Relation of cyclosporine blood levels to adverse effects on lipoproteins. *Transplantation* **57:** 1479-1483 (1994).

105. A. J. Humberstone, C. J. H. Porter, G. A. Edwards, and W. N. Charman. Post-prandial hypertriglyceridemia affects the pharmacokinetics of

halofantrine after intravenous administration to beagle dogs. *Pharm.Res.* **12:** S356 (1995).

106. J. Wojcicki, W. Kalinowski, and B. Gawronski-Szklarz. Comparative pharmacokinetics of doxycycline and oxytetracycline in patients with hyperlipidemia. *Arzneim.Forsch.* **35:** 991-993 (1985).

107. G. Dutkiewicz, J. Wojcicki, and B. Gawronski-Szklarz. Pharmacokinetics of phenytoin in hyperlipidemia. *Eur.J.Pharm.Sci.* **4:** 33-37 (1996).

108. D. Luke. Immunosuppressive effect of cyclosporine in the hyperlipidemic rat model. *Biopharm.Drug Dispos.* **13:** 635-645 (1992).

109. J. Nemunaitis, H. J. Deeg, and G. C. Yee. High cyclosporine levels after bone marrow transplantation associated with hypertriglyceridemia. *Lancet* **Sept. 27:** 744-745 (1986).

110. M. Nishimura, K. Yamada, K. matsushita, K. Sakamotp, T. Saisu, H. Kashiwabara, and T. Yokoyama. Changes in trough levels of whole blood cyclosporine and graft function of a kidney transplant recipient with onset of hypothyroidism after transplantation. *Transplantation* **62:** 1509-1511 (1996).

111. R. Wassef, Z. Cohen, and B. Langer. In vivo interaction of cyclosporine and intralipid. *Transplantation* **41:** 266-268 (1986).

112. N. De Kippel, J. Sennesael, J. Lamote, G. Ebinger, and J. De Keyser. Cyclosporine leukoencephalopathy induced by intravenous lipid solution. *Lancet* **339:** 1114-1115 (1992).

113. W. N. Awni, B. L. Kasiske, K. Heim-Duthoy, and R. K. Vennkateswara. Long-term cyclosporine pharmacokinetic changes in renal transplant recipients. *Clin.Pharmacol.Ther.* **45:** 41-48 (1989).

114. S. K. Gupta and L. Z. Benet. High-fat meals increase the clearance of cyclosporine? *Pharm.Res.* **7:** 46-48 (1990).

115. S. K. Gupta, R. C. Manfro, Tomlanovich.S.J., J. G. Gambertoglio, M. R. Garovoy, and L. Z. Benet. Effect of food on the pharmacokinetics of cyclosporine in healthy subjects following oral and intravenous administration. *J.Clin.Pharmacol.* **30:** 643-653 (1990).

116. K. M. Wasan and J. S. Conklin. Enhanced amphotericin B nephrotoxicity in intensive care patients with elevated levels of low-density lipoprotein cholesterol. *Clin.Infect.Dis.* **24:** 78-80 (1997).

117. K. M. Wasan. Modifications in plasma lipoprotein concentration and lipid composition regulate the biological activity of hydrophobic drugs. *J.Pharmacol.Toxicol.* **36:** 1-11 (1996).

118. J. Brajtburg, S. Elberg, J. Bolard, G. Kobayaski, R. Levy, R. Ostlund, D. Schlessinger, and G. Medoff. Interaction of plasma proteins and lipoproteins with amphotericin B. *J.Infect.Dis.* **149:** 986-997 (1984).

119. P. Chavanet, V. Joly, D. Rigaud, J. Bolard, C. Carbon, and P. Yeni. Influence of diet on experimental toxicity of amphotericin B deoxycholate. *Antimicrob.Agents Chemother.* **38:** 963-968 (1994).

120. K. M. Wasan, M. G. Rosenblum, L. Cheung, and G. Lopez-Berestein. Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. *Antimicrob.Agent.Chemother.* **38:** 223-227 (1994).

121. K. M. Wasan, G. A. Brazeau, A. Keyhani, A. C. Hayman, and G. Lopez-Berestein. Role of liposome composition and temperature in distribution of amphotericin B in serum lipoproteins. *Antimicrob.Agents Chemother.* **37:** 246-250 (1993).

122. K. M. Wasan, P. H. Pritchard, M. Ramaswamy, W. Wong, E. M. Donnachie, and L. J. Brunner. Differences in lipoprotein lipid concentration and composition modify the plasma distribution of cyclosporine. *Pharm.Res.* **14:** 1613-1620 (1997).

123. K. M. Wasan, M. Ramaswamy, M. P. McIntosh, C. J. H. Porter, and W. N. Charman. Differences in lipoprotein concentration and lipid transfer protein I activity modify the in vitro plasma distribution of halofantrine in dyslipidemic plasma. *Pharm.Res.* **14:** S277 (1997).

124. S. Urien. Interaction of drugs with human plasma lipoproteins. In J. P. Tillement and E. Lindenlaub (eds.), *Proceedings of the symposium on*

protein binding and drug transport., Algavre:F. K. Schatauer Verlag, Stuttart-New York, 1986, pp. 63-75.

125. M. H. Koldin, G. S. Kobayaski, J. Brajtburg, and G. Medoff. Effects of elevation of serum cholesterol and administration of amphotericin B complexed to lipoproteins on amphotericin B-induced toxicity to rabbits. *Antimicrob.Agents Chemother.* **28:** 144-145 (1985).

126. T. Prueksaritanont, M. Koike, B.-A. Hoener, and L. Z. Benet. Transport and metabolism of cyclosporine in isolated rat hepatocytes. *Biochem.Pharmacol.* **43:** 1997-2006 (1992).

127. T. A. Ho Ngoc-Ta and G. Sirois. Competitive binding of quinidine, propranolol, and 4-OH-propranolol to human plasma very low-density (VLDL) and low-density (LDL) lipoproteins. *Pharm.Acta Helv.* **60:** 106-109 (1985).

128. G. Sager, O. G. Nilsen, and S. Jacobsen. Variable binding of propranolol in human serum. *Biochem.Pharmacol.* **28:** 905-911 (1979).

129. H. Rosenkranz, K. Schlossmann, and W. Scholtan. [The binding of 4-(2'-nitrophenyl)-2,6-dimethyl-1,4- dihydropyridine-3,5-dicarboxylic acid dimethyl ester (nifedipine) as well as other coronary active substances to serum albumins]. [German]. *Arzneimittelforschung* **24:** 455-466 (1974).

130. J. L. Pinquier, S. Urien, M. Lemaire, P. Chaumet-Riffaud, and J. P. Tillement. Comparative binding of two closely related dihydropyridines

(isradipine and darodipine) to serum proteins and erythrocytes. *Pharmacology* **36:** 305-312 (1988).

131. P. R. Cullis and M. J. Hope. Physical properties and functional roles of lipids in membranes. In D. E. Vance and J. E. Vance (eds.), *Biochemistry of lipids and membranes*, The Benjamin/Cummings Publishing Co, Inc., Menlo Park, California, 1985, pp. 25-71.

132. H. Garda and R. Brenner. In vitro modification of cholesterol content of rat liver microsomes. Effects upon membrane 'fluidity' and activities of glucose-6-phosphatase and fatty acid desaturation systems. *Biochim.Biophys.Acta* **819:** 45-54 (1998).

133. F. Feo, R. A. Canuto, R. Garcea, and O. Brossa. The role of lipid-protein interactions in NADPH-cytochrome c reductase of rat liver mitochondria. *Biochim.Biophys.Acta* **504:** 1-14 (1978).

134. E. M. L. Bastiaanse, K. M. Hold, and A. Van der Laarse. The effect of membrane cholesterol content on ion transport processes in plasma membranes. *Cardiovascular Research* **33:** 283 (1997).

135. S. Imaoka, Y. Imai, T. Shimada, and Y. Funae. Role of phospholipids in reconstituted cytochrome P450 3A form and mechanism of their activation of catalytic activity. *Biochemistry* **31:** 6063-6069 (1992).

136. M. M. Gleason, M. S. Medow, and T. N. Tulenko. Excess membrane cholesterol alters calcium movements, cytosolic calcium levels, and membrane fluidity in arterial smooth muscle cells. *Circ.Res.* **69:** 227 (1991).

137. A. Plewka and M. Kaminiski. Influence of cholesterol and protein diet on liver cytochrome P-450-dependent monooxygenase system in rats. *Exp.Toxic.Pathol.* **48:** 249-253 (1996).

138. M. Murray. P450 enzymes. Inhibition mechanisms, genetic regulation and effects of liver disease. *Clin.Pharmacokinet.* **23:** 132-146 (1992).

139. L. L. Moltke, D. J. Greenblatt, J. Schmider, J. S. Harmatz, and R. I. Shader. Metabolism of drugs by cytochrome P450 3A isoforms. *Clin.Pharmacokinet.* **20:** 1006 (1995).

140. A. T. Remaley, U. K. Schumacher, H. R. Amouzadeh, B. Brewer, and J. M. Hoeg. Identification of novel differentially expressed hepatic genes in cholesterol-fed rabbits by a non-targeted gene approach. *J.Lip.Res.* **36:** 308-314 (1995).

141. A. Irizar, CR. Barnett, PR. Flatt, and C. Ioannides. Defective expression of cytochrome P450 proteins in the liver of the genetically obese Zucker rat. *Eur.J.Pharmacol-Environ.Toxicol.Pharmacol.Sec.* **293:** 385-393 (1995).

142. M. Watanabe, H. Nakura, T. Tateishi, M. Tanaka, T. Kumai, and S. Kobayashi. Reduced hepatic drug-metabolizing enzyme activities in

spontaneously hyperlipidaemic rat as an animal model of hypercholesterolaemia. *Med.Sci.Res.* **24:** 257-259 (1996).

143. S. Rubattu, R. Russo, B. Gigante, A. F. L. Porcellini, B. Trimarco, and M. Volpe. Regulation of 11-beta-hydroxylase cytochrome P450 expression by cholesterol in spontaneously hypertensive rats. *J.Hypertens.* **13:** 1253-1258 (1995).

144. R. Ferrari. Major differences among the three classes of calcium antagonists. *Eur.Heart J.* **18:** A56-A70 (1997).

145. W. A. Catterall and J. Striessig. Receptor sites for calcium channel antagonists. *TiPS* **13:** 256-261 (1992).

146. T. Godfraind. Calcium antagonists and vasodilation. *Pharmac.Ther.* **64:** 37-75 (1994).

147. W. Rafflenbeul. Nifedipine in acute coronary syndromes: Furberg's refrain revisited. *European Heart Journal* **17:** 1147-1152 (1996).

148. H. S. Mueller and R. A. Chahine. Interim report of multicenter double-blind, placebo-controlled studies of nifedipine in chronic stable angina. *Am.J.Med* **71:** 645-657 (1981).

149. P. A. Poole-Wilson. The calcium antagonist controversy; implications beyond drug prescription. *Eur.Heart J.* **17:** 1131-1133 (1996).

150. J. G. P. Tijssen and P. G. Hugenholtz. Critical appraisal of recent studies on nifedipine and other calcium channel blockers in coronary artery disease and hypertension. *European Heart Journal* **17:** 1152-1157 (1996).

151. C. D. Furberg, B. M. Psaty, and J. V. Meyer. Nifedipine. Dose-related increase in mortality in patients with coronary heart disease. *Circulation* **92:** 1326-1331 (1995).

152. C. D. Furberg, M. Pahor, and B. M. Psaty. The unnecessary controversy. *Eur.Heart J.* **17:** 1147 (1996).

153. Holland Inter-university Nifedipine/metoprolol Trial (HINT) Research Group. Early treatment of unstable angina in the coronary care unit: a randomized double-blind, placebo-controlled comparison of recurrent ischaemia in patients treated with nifedipine or metoprolol or both. *Br.Heart J.* 400-413 (1986).

154. The Israeli SPRINT Study Group. Secondary Prevention Reinfarction Israeli Nifedipine Trial (SPRINT): a randomized intervention trial of nifedipine in patients with acute myocardial infarction. *Eur.Heart J.* **9:** 354-364 (1988).

155. M. C. Isordia, A. Chavez-Negrete, J. A. Palma-Aguirre, F. J. Flores-Murrieta, and G. Castaneda-Hernandez. Pharmacokinetics and pharmacodynamic differences between nifedipine capsule and GITS formulations in hypertensive patients. *Int.J.Clin.Pharmacol.Ther.* **35:** 133 (1997).

156. G. Castaneda-Hernandez, C. Hoyo-Vadillo, and J. E. Herrera. Differences in nifedipine concentration-effect relationship between capsule and slow release tablet administration. *Int.J.Cardiol.* **33:** 56-60 (1995).

157. R. Donnelly, H. L. Elliot, and P. A. Meredith. Concentration-effect analysis of antihypertensive drug response. Focus on calcium antagonists. *Clin.Pharmacokinet.* **26:** 472-485 (1994).

158. L. H. Opie. New developments in the calcium channel antagonist controversy. *Circulation* **95:** 1668-1669 (1997).

159. S. Yamashita. Current controversies in calcium channel blocker therapy. *Can.J.Clin.Pharmacol.* **3:** 99-108 (1996).

160. P. R. Lichtlen, P. G. Hugenholtz, W. Rafflenbeul, H. Hecker, S. Jost, J. W. Deckers, and on behalf of the INTACT group investigators. Retardation of angiographic progression of coronary artery disease by nifedipine: results of the Internations Nifedipine Trial of Antiatherosclerotic Therapy (INTACT). *Lancet* **335:** 1113 (1990).

161. J. Russell, D. Koeslag, P. Dolphin, and R. Amy. Prevention of myocardial lesions of JCR:LA-corpulent rats by nifedipine. *Arteriosclerosis* **10:** 658-664 (1990).

162. S. L. Ali. *Nifedipine. In: Analytical Profiles of Drug Substances (Ed.: K. Florey)*, Academic Press, New York, NY, 221-288, 1989.

163. R. Mannhold, R. Rodenkirchen, R. Bayer, and W. Haas. The importance of drug ionization for the action of calcium antagonists and related compounds. *Arzneimittelforschung* **34:** 407-409 (1984).

164. J. S. Grundy, R. Kherani, and R. T. Foster. Photostability determination of commercially available nifedipine oral dosage formulations. *J.Pharm.Biomed.Anal.* **12:** 1529-1535 (1994).

165. T. S. Foster, S. R. Hamann, V. R. Richards, P. J. Bryant, D. A. Graves, and R. G. McAllister. Nifedipine kinetics and bioavailability after single intravenous and oral doses in normal subjects. *J.Clin.Pharmacol.* **23:** 161-170 (1983).

166. T. J. Rashid, U. Martin, H. Clarke, D. G. Waller, A. G. Renwick, and C. F. George. Factors affecting the absolute bioavailability of nifedipine. *Br.J.Clin.Pharmacol.* **40:** 51-58 (1995).

167. N. Holtbecker, M. F. Fromm, H. K. Kroemer, E. E. Ohnhaus, and H. Heidemann. The nifedipine-rifampin interaction. Evidence for induction of gut wall metabolism. *Drug Metab.Dispos.* **24:** 1121-1123 (1996).

168. S. Tsuruta, K. Nakamura, K. Arimori, and M. Nakano. Inhibition of nifedipine metabolism in dogs by erythromycin: Difference between the gut wall and the liver. *J.Pharm.Pharmacol.* **49:** 1205-1210 (1997).

169. J. S. Grundy, L. A. Eliot, and R. T. Foster. Extrahepatic first-pass metabolism of nifedipine in the rat. *Biopharm.Drug Dispos.* **18:** 509-522 (1997).

170. D. A. Smith. Species differences in metabolism and pharmacokinetics: Are we close to an understanding? *Drug Metab.Rev.* **23:** 355-373 (1991).

171. J. Otto and L. J. Lesko. Protein binding of nifedipine. *J.Pharm.Pharmacol.* **38:** 399-400 (1986).

172. C. H. Kleinbloesem, P. van Brummelen, J. van Harten, M. Danhof, and D. D. Breimer. Nifedipine: influence of renal function on pharmacokinetic/hemodynamic relationship. *Clin.Pharmacol.Ther.* **37:** 563-574 (1985).

173. C. H. Kleinbloesem, J. van Harten, J. P. Wilson, M. Danhof, P. van Brummelen, and D. D. Breimer. Nifedipine: kinetics and hemodynamic effects in patients with liver cirrhosis after intravenous and oral administration. *Clin.Pharmacol.Ther.* **40:** 21-28 (1986).

174. D. R. C. Robertson, D. G. Waller, A. G. Renwick, and C. F. George. Age-related changes in the pharmacokinetics and pharmacodynamics of nifedipine. *Br.J.Clin.Pharmacol.* 297-305 (1988).

175. C. H. Kleinbloesem, P. van Brummelen, and D. D. Breimer. Nifedipine. Relationship between pharmacokinetics and pharmacodynamics. [Review]. *Clin.Pharmacokinet.* **12:** 12-29 (1987).

176. B. Cenni, J. Meyer, R. Brandt, and B. Betschart. The antimalarial drug halofantrine is bound mainly to low and high-density lipoproteins in human serum. *Br.J.Clin.Pharmacol.* **39:** 519-526 (1995).

177. Dihydropyridine calcium antagonists and the trough:peak ratio focus on adverse effects. *J.Hypertens.* **12:** S73-S77 (1994).

178. C. H. Kleinbloesem, P. van Brummelen, H. Faber, M. Danhof, N. P. Vermeulen, and D. D. Breimer. Variability in nifedipine pharmacokinetics and dynamics: a new oxidation polymorphism in man. *Biochem.Pharmacol.* **33:** 3721-3724 (1984).

179. R. M. Kacmarek, C. W. Mac, and S. Dimas. Hemodynamic monitoring. In R. M. Kacmarek, C. W. Mac, and S. Dimas (eds.), *The Essentials of Respiratory Therapy.*, Medical Book Inc., Chicago, 1985,

180. J. S. Grundy, R. Kherani, and R. T. Foster. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. *J.Chromatogr.B Biomed.Appl.* **654:** 146-151 (1994).

181. H. J. Baker, J. R. Lindsey, and S. H. Weisbroth. Selected Normative Data. *The Laboratory Rat. Volume I. Biology and Disease*, Academic Press, New York, 1979, pp. 411.

182. R. H. Muller. Toxicity studies in hepatocyte cultures. *Colloidal Carriers for Controlled Drug Delivery and Targeting*, CRC Press, Boca Raton, 1991, pp. 207-210.

183. A. Corsini, M. Raiteri, M. R. Soma, F. Bernini, R. Fumagalli, and R. Paoletti. Pathogenesis of atherosclerosis and the role of 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitors. *Am.J.Cardiol.* **76:** 21A-28A (1995).

184. Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, relative weight and ECG abnormalities to incidence of major coronary events: Final report of the Pooling Project. *J.Chron.Dis.* **31:** 201-306 (1978).

185. J. M. Barnes and F. A. Denz. Experimental methods used in determining chronic toxicity. A critical review. *Pharmacol.Rev.* **6:** 191-242 (1954).

186. D. Scherling, W. Karl, M. Radtke, H. J. Ahr, and H.-M. Siefert. Biotransformation of nifedipine in rat and dog. *Arzneimittelforschung* **42:** 1292-1300 (1992).

187. D. R. Klaus and S. Johannes. Pharmacokinetics and metabolism of nifedipine. *Hypertension* **5:** 18-24 (1983).

188. P. A. Meredith and H. L. Elliott. FDA guidelines on trough:peak ratios in the evaluation of antihypertensive agents. *J.Cardiovasc.Pharmacol.* **23:** S26-S30 (1994).

189. H. Lithell, B. Odlind, I. Selinus, A. Lindberg, B. Lindstrom, and L. Frodin. Is the plasma lipoprotein pattern of importance for treatment with cyclosporine? *Transplant.Proc.* **18:** 50-51 (1986).

190. M. Piquette-Miller, A. Pak, H. Kim, R. Anari, and A. Shahzamani. Decreased Expression and Activity of P-Glycoprotein in Rat Liver During Acute Inflammation. *Pharm Res* **15:** 706-711 (1998).

191. F. J. Sharom. The P-glycoprotein multidrug resistance transporter: interactions with membrane lipids, and their modulation of activity. *Biochemical Society Transcations* **25:** 1088-1097 (1997).

192. M. E. Sheridan, G. S. Clarke, and M. L. Robinson. Analysis of nifedipine in serum using solid-phase extraction and liquid chromatography. *J.Pharm.Biomed.Anal.* **7:** 519-522 (1989).

193. L. A. Eliot, R. T. Foster, and F. Jamali. Effects of hyperlipidemia on the pharmacokinetics of nifedipine. *Pharm Res* **Accepted:** 1998).

194. A. Endo, Y. Tsujita, M. Kuroda, and K. Tanzawa. Effects of ML-236B on cholesterol metabolism in mice and rats: Lack of hypocholesterolemic activity in normal animals. *Biochim.Biophys.Acta* **575:** 266-276 (1979).

195. S. Kasim, R. LeBoeuf, S. Khilnani, L. Tallapaka, D. Dayananda, and C. Jen. Mechanisms of triglyceride-lowering effect of an HMG-CoA reductase inhibitor in a hypertrigyceridemic animal model, Zucker obese rat. *Journal of Lipid Research* **33:** 1-7 (1992).

196. W. G. Linscheer, B. Atreyee, K. M. Uma, W. John, N. Sandor, and N. Jyotirmoy. Lovastatin induces synthesis of cholesterol, which acts as a

secretagogue of biliary phopholipids in rats. *Am.J.Physiol.* **268:** G242-G250 (1995).

197. R. Fears, D. H. Richards, and H. Ferres. The effect of compactin, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase activity, on cholesterogenesis and serum cholesterol levels in rats and chicks. *Atherosclerosis* **35:** 439-449 (1980).

198. T. Hirano, F. Komuro, S. Furukawa, S. Nagano, and T. Takahashi. Effect of pravastatin sodium, a new inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A reductase, on very-low-density lipoprotein composition and kinetics in hyperlipidemia associated with experimental nephrosis. *Metabolism* **39:** 605-609 (1990).

199. Y. Tsujita, M. Kuroda, and Y. Shimada. CS-514, a competitive inhibitor of 3-hydrox-3-methylglutaryl coenzyme A reductase: Tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. *Biochim.Biophys.Acta* **877:** 50-60 (1986).

200. B. R. Krawuse and R. S. Newton. Lipid-lowering activity of atorvastatin and lovastatin in rodent species: triglyceride-lowering in rats correlates with efficacy in LDL animal models. *Atherosclerosis* **117:** 244 (1995).

201. J. D. Bergstrom, R. G. Bostedor, D. J. Rew, W. M. Geissler, S. D. Wright, and Y.-S. Chao. Hepatic responses to inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase: A comparison of atorvastatin and

simvastatin. *Biochimica et Biophysica Acta - Lipids & Lipid Metabolism.* **1389:** 213-221 (1998).

202. W. G. Linscheer, B. Atreyee, K. M. Uma, W. John, N. Sandor, and N. Jyotiromoy. Lovastatin induces synthesis of cholesterol, which acts as a secretagogue of biliary phospholipids in rats. *Am.J.Physiol.* **268:** 31-32 (1995).

203. M. S. Brown, J. R. Faust, J. L. Goldstein, I. Kaneko, and A. Endo. Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B), a competitive inhibitor of the reductase. *J.Biol.Chem.* **253:** 1121 (1978).

204. W. R. Bensch, T. S. Ingebritsen, and E. R. Diller. Lack of correlation between the rate of cholesterol biosynthesis and the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in rats and in fibroblasts treated with ML-236B. *Biochem.Biophys.Res.Commun.* **82:** 247 (1978).

205. B. Roth, C. J. Blankley, A. W. Chucholowski, E. Ferguson, D. F. Ortwine, R. S. Newton, C. S. Sekerke, D. R. Sliskovic, C. D. Stratton, and M. W. Wilson. Inhibitors of cholesterol biosynthesis. *J.Med.Chem.* **34:** 357 (1991).

206. D. R. Sliskovic, J. A. Picard, W. H. Roark, B. D. Roth, E. Ferguson, B. R. Krause, R. S. Newton, C. Sekerke, and M. K. Shaw. Inhibitors of cholesterol biosynthesis, a novel series of HMG-CoA reductase inhibitors. *J.Med.Chem.* **34:** 367 (1991).

207. M. K. Shaw, R. S. Newton, D. R. Sliskovic, B. D. Roth, E. Ferguson, and B. R. Krause. HegG2 cells and primary rat hepatocytes differ in their response to inhibitors of HMG-CoA reductase. *Biochem.Biophys.Res.Commun.* **170:** 726 (1990).

208. G. D. Clifton, D. C. Booth, S. Hobbs, B. A. Boucher, T. S. Foster, R. G. McAllister, and A. N. DeMaria. Negative inotropic effect of intravenous nifedipine in coronary artery disease: Relation to plasma levels. *Am.Heart J.* **119:** 1990).

209. F. I. Achike and S. Dai. Cardiovascular responses to verapamil and nifedipine in hypoventilated and hyperventilated rats. *Br.J.Pharmacol.* **100:** 102-106 (1990).

210. B. V. Reed and R. Tuma. The effects of nifedipine on regional vascular resistance and blood flow in the spontaneously hypertensive rat. *Clin.Exp.Hypertens.* **8:** 963-979 (1986).

211. F. P. Guengerich, M. V. Martin, P. H. Beaune, P. Kremers, T. Wolff, and D. J. Waxman. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J.Biol.Chem.* **261:** 5051-5060 (1986).

212. G. Dutkiewicz, J. Wojcicki, and B. Gawronska-Szklarz. Effect of hyperlipidemia on pharmacokinetics of free phenytoin. *Neur.Neurochir.Pol.* **28:** 203-211 (1994).

213. A. Gurusinghe, M. de Niese, J. F. Renaud, and L. Austin. The binding of lipoproteins to human muscle cells: binding and uptake of LDL, HDL, and alpha-tocopherol. *Muscle Nerve* **11:** 1231-1239 (1988).

214. K. Vadiei, G.Lopez-Berestein, and D. R. Luke. Disposition, and toxicity of amphotericin-B in the hyperlipidemic Zucker rat model. *Int.J.Obesity* **14:** 465-472 (1990).

215. J. J. Vallner and Chen.L. Beta-lipoproteins:possibly plasma transport proteins for basic drugs. *J.Pharm.Sci.* **66:** 420-421 (1977).

216. M. U. S. Lemaire, E. Albengres, P. Riant, R. Zini, and J.-P. Tillement. Lipoprotein binding of drugs. In M. M. Reidenberg and S. Erill (eds.), *Drug-Protein Binding*, Praeger, Newy York, 1984, pp. 93-108.

217. In F. W. Putnam (ed.). Serum Protein. In F. W. Putnam (ed.), *The Plasma Proteins*, Academic Press, New York, 1975, pp. 133-138.

218. Rajaram O.V., P. Fatterpaker, and A. Sreenivasan. Involvement of binding lipoproteins in the absorption and transport of alpha-tocopherol in the rat. *Biochem.J.* **140:** 516 (1974).

219. S. Urien, P. Riant, E. Albengres, R. Brieude, and J.-P. Tillement. In vitro studies on the distribution of probucol among human plasma lipoproteins. *Molecular Pharmacol.* **26:** 327 (1984).

220. M. Lemaire, W. M. Pardridge, and G. Chaudhuri. Influence of blood components on the tissue uptake indices of cyclosporine in rats. *J.Pharmacol.Exp.Ther.* **244:** 740-743 (1988).

221. J. S. Clements and J. E. Peacock. Amphotericin B revisited:reassessment of toxicity. *Am.J.Med.* **88:** 22N-27N (1990).

222. A. Vertut-Croquin, J. Bolard, M. Chabbert, and C. Gary-Bobo. Differences in the interaction of the polyene antibiotic amphotericin B with cholesterol- or ergosterol-containing phospholipid vesicles. A circular dichroism and permeability study. *Biochem.* **22:** 2939-2940 (1983).

223. J. D. Readio and R. Bittman. Equilibrium binding of amphotericin B and its methyl ester and borate complex to sterols. *Biochim.Biophys.Acta* **685:** 219-224 (1982).

224. K. M. Wasan, R. E. Morton, M. G. Rosenblum, and G. Lopez-Berestein. Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high-density lipoprotein:role of lipid transfer protein. *J.Pharm.Sci.* **83:** 1006-1010 (1994).

225. K. S. Pang and M. Rowland. Hepatic clearance of drugs. I.Theoretical considerations of a "Well-Stirred" model and a "Parallel Tube" model.

Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J.Pharmacokinet.Biopharm.* **5:** 625-653 (1977).

226. J. A. Jansen. Influence of plasma protein binding kinetics on hepatic clearance assessed from a "tube" model and a "well-stirred" model. *J.Pharmacokinet.Biopharm.* **9:** 15-26 (1981).

227. D. Shand, R. Cotham, and G. Wilkinson. Perfusion-limited effects of plasma drug binding on hepatic drug extraction. *Life Sci.* **19:** 125-130 (1976).

228. G. C. Yee and D. R. Salomon. Cyclosporine. In W. E. Evans, J. J. Schentag, and W. J. Jusko (eds.), *Applied Pharmacokinetics. Principle of therapeutic drug monitoring.*, Applied Therapeutic, Inc., Ann Arbor, 1992, pp. 28-1.

229. W. N. Awni and R. J. Sawchuk. The pharmacokinetics of cyclosporine. II. Blood plasma binding and distribution. *Drug Metab.Dispos.* **13:** 133-138 (1985).

230. A. J. Wood, G. Maurer, W. Niederberger, and T. Beveridge. Cyclosporine: pharmacokinetics, metabolism, and drug interactions. *Transplant.Proc.* **15:** 2409-2412 (1983).

231. B. Legg, S. K. Gupta, and M. Rowland. A model to account for the variation in cyclosporine binding to plasma lipids in transplant patients. *Ther.Drug Monit.* **10:** 20-27 (1988).

232. C. H. Kleinbloesem, P. Brummelen, H. Faber, and D. D. Breimer. Pharmacokinetics and hemodynamic effects of long-term nifedipine treatment in hypertensive patients. *J.Cardiovasc.Pharmacol.* **9:** 202-208 (1987).

233. K. H. Graefe, R. Ziegler, W. Wingender, K. Ramsch, and H. Schmitz. Plasma concentration-response relationships for some cardiovascular effects of dihydropyridines in healthy subjects. *Clin.Pharm.Ther.* **43:** 16-22 (1988).

234. A. G. Renwick, D. R. Robertson, B. Macklin, V. Challenor, D. G. Waller, and C. F. George. The pharmacokinetics of oral nifedipine--a population study. *Br.J.Clin.Pharmacol.* **25:** 701-708 (1988).

235. P. A. Soons, H. C. Schoemaker, A. F. Cohen, and D. D. Breimer. Intraindividual variability in nifedipine pharmacokinetics and effects in healthy subjects. *J.Clin.Pharmacol* **32:** 324-331 (1992).

236. G. Mancia and P. A. Zwieten. How safe are calcium antagonists in hypertension and coronary heart disease? *J.Hypertens.* **14:** 13-17 (1996).

237. P. A. van Zwieten and M. Pfaffendorf. Developments and trends in the field of calcium antagonists. *High Blood Press.* **3:** 356-366 (1994).

238. D. E. Hilleman, S. M. Mohiuddin, B. D. Lucas, Jr., B. Shinn, and G. N. Elsasser. Conversion from sustained-release to immediate-release calcium

entry blockers: outcome in patients with mild-to-moderate hypertension. *Clin.Ther.* **15:** 1002-1010 (1993).

239. C. Campana, I. Iacona, M. B. Regazzi, A. Gavazzi, G. Perani, V. Raddato, C. Montemartini, and M. Vigano. Efficacy and pharmacokinetics of simvastatin in heart transplant recipients. *Ann.Pharmacother.* **29:** 235-239 (1995).

240. L. Gullestad, Norda.K.P., K. Forfang, H. Ihlen, A. Hostmark, K. J. Berg, H. Cheng, M. S. Schwartz, O. Geiran, and S. Simonsen. Post-transplant hyperlipidaemia: low-dose lovastatin lower atherogenic lipids without plasma accumulation of lovastatin. *J.Intern.Med.* **242:** 490 (1997).

241. M. E. Rosenfeld, A. Chait, E. L. Bierman, W. King, P. Goodwin, C. E. Walden, and R. Ross. Lipid composition of aorta of Watanabe heritable hyperlipemic and comparable hypercholesterolemic fat-fed rabbits. Plasma lipid composition determines aortic lipid composition of hypercholesterolemic rabbits. *Arteriosclerosis* **8:** 338-347 (1988).

242. K. Tanzawa, Y. Shimada, M. Kuroda, Y. Tsujita, M. Arai, and Y. Watanabe. WHHL rabbit: a low density lipoprotein receptor deficient animal model for familial hypercholesterolemia. *FEBS Lett.* **118:** 81-84 (1980).

243. D. R. Luke, K. Vadiei, and L. J. Brunner. Influence of circadian variations in triglyceride production and/or clearance on cyclosporine pharmacokinetics and toxicity. *Ann.Rev.Chronopharmacol.* **6:** 285 (1988).