Effects of Hyperlipidemia on the Pharmacokinetics of Nifedipine in the Rat

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Received August 3, 1998; accepted November 3, 1998

Purpose. The effect of hyperlipidemia on nifedipine pharmacokinetics was studied. The mechanisms by which hyperlipidemia affects pharmacokinetics of drugs are mainly undetermined. Hyperlipidemia may decrease the fraction of unbound drug in plasma and/or decrease intrinsic ability of the cytochrome P-450 systems due to excess membrane cholesterol. Hyperlipidemia is a primary risk factor for coronary artery disease leading to hypertension and ischemic heart disease, for which nifedipine, a calcium channel blocker, is used.

Methods. Poloxamer 407 (P407)-induced hyperlipidemic rat model was used to study the effects of hyperlipidemia on the pharmacokinetics of nifedipine (6 mg kg^{-1} given iv, ip and po). Total plasma cholesterol levels increased from 0.82–2.02 to 5.27–11.05 mmol L^{-1} 48 h post P407 administration (1g kg^{-1}, ip). Protein binding studies were conducted by an ultrafiltration method.

Results. Hyperlipidemia significantly decreased CL_{TB} by 38% and CL_{TB}/F by 45 and 42% following po and ip doses, respectively, thereby increasing $AUC_{0-\infty}$, C_{max} and half-life. Absolute bioavailability and Vd_{ss} remained unchanged. $AUC_{0-\infty}$ was affected to the same extent in each route of administration, therefore, the effect was mainly systemic rather than presystemic. Hyperlipidemia significantly lowered the fraction unbound in plasma by approximately 31%.

Conclusions. The altered pharmacokinetics of nifedipine by P407-induced HYPERLIPIDEMIA may be, at least in part, due to the decrease in fraction unbound in plasma. A decrease in intrinsic clearance, however, cannot be ruled out.

KEY WORDS: hyperlipidemia; hypercholesterolemia; nifedipine; pharmacokinetics; protein binding; rat.

INTRODUCTION

Hyperlipidemia is an abnormal elevation in one or more of the plasma lipids; cholesterol, triglycerides and phospholipids (1). Hyperlipidemia influences the pharmacokinetics and pharmacodynamics of many drugs, however, the mechanism(s) involved remains unresolved. The plasma lipids are carried in the form of lipoproteins. Low density lipoproteins (LDL) are the major cholesterol carrying lipoproteins accounting for 60–70% of total cholesterol in human. High density lipoproteins (HDL) and very low density lipoproteins (VLDL) account for approximately 20–30% and 10–15%, respectively. Lipoproteins differ in density, lipid and protein composition, metabolism, and mechanism of action, and may, therefore, have different effects on drug disposition (1). The variation in lipoprotein concentration is large within the normal population.

Several lipophilic drugs bind to lipoproteins with more affinity and capacity as compared to albumin and α_1-acid glycoprotein (AAG) (2). Pathological changes in lipoprotein concentrations may therefore alter binding affinity and/or capacity. In addition, in vivo studies have shown that increased levels of cholesterol may decrease cytochrome P450 functionality (3). A change in the pharmacologically active free fraction or intrinsic clearance may result in altered metabolism and/or clinical response in these patients. Hyperlipidemia is one of the primary causes of atherosclerosis, often resulting in hypertension and coronary heart disease. Nifedipine is a lipophilic calcium channel blocker and a potent vasodilator, which is widely used in the treatment of ischaemic heart disease and hypertension. In vitro studies have shown that nifedipine binds to albumin, AAG and lipoproteins (4). The purpose of this study was to determine the effects of hyperlipidemia on the pharmacokinetics of nifedipine.

MATERIALS AND METHODS

Animal Treatment and Dosing

Male Sprague-Dawley rats weighing between 260–340 g were obtained from Biosciences Animal Service (University of Alberta, Edmonton, AB, Canada) and procedures followed the ethics of animal investigation. They were grouped to receive either a 1 ml saline or 1 g kg^{-1} poloxamer 407 (P407) (Pluronic F127, BASF Corporation Canada Inc., Toronto, ON, Canada) by intraperitoneal (ip) injection resulting in either normolipidemic or hyperlipidemic rats, respectively. The animals were anesthetised with 65 mg kg^{-1} of sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, ON, Canada) and jugular vein cannulation was completed to provide a site for blood sampling. In addition, a midline incision into the lower abdomen was made to ensure proper administration of P407 into the intraperitoneal cavity. Surgery is required since P407 cannot be administered by needle due to the thickness of the gel. P407 (30% w/v) solution was prepared by the cold method (5) 24 h prior to use and was kept at 2–5°C in order to maintain a liquid phase. Needles and syringes were cooled prior to P407 injection to prevent gelation within the syringe. Food and water was given ad libitum throughout the entire study. Forty-eight hours after the surgical procedures and administration of P407 the animals were given nifedipine (Sigma, Canada Mississauga, ON, Canada) 6 mg kg^{-1} which was dissolved in polyethylene glycol 400 (5 mg ml^{-1}) either by a 4 min iv infusion, intraperitoneal (ip) injection or oral (po) gavage. Blood samples of approximately 150–250 μl were drawn through the jugular vein

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ABBREVIATIONS: AAG, α_1-acid glycoprotein; $AUC_{(0-\infty)}$, area under the plasma concentration-time curve from 0 min to infinity; $AUC_{(0-last)}$, area under the plasma concentration-time curve from 0 min to the last measurable concentration; $AUMC_{(0-last)}$, area under the first moment plasma concentration-time curve from 0 min to the last measurable concentration; C_{max}, maximum concentration; CL_{TB}, total body clearance; CL_{TB}/F, intraperitoneal or oral clearance; F, absolute bioavailability; f_{up}, fraction unbound in plasma; f_{ut}, fraction unbound in tissue; HDL, high density lipoproteins; iv, intravenous; ip, intraperitoneal; LDL, low density lipoproteins; po, oral; P407, Poloxamer 407, Pluronic F127; $t_{1/2}$, half life; t_{max}, time of maximum concentration; Vdss, Volume of distribution at steady state; VLDL, Very low density lipoprotein; Vp, Volume of plasma; Vt, Volume of tissue; λ, elimination rate constant.

0724-8741/99/0200-0309$16.00/0

cannula with heparinized syringes. Saline was administered through the jugular vein cannula immediatley after blood sampling to replace the lost blood. To prevent nifedipine degradation blood and plasma samples were collected and handled in a room illuminated only by sodium light. The samples were frozen at −20°C until analyzed.

The iv bolus dose was injected slowly from 0 to 4 min through the jugular vein cannula which was rinsed thoroughly with blood and saline prior to blood sampling at 0, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180 and 240 min after start of infusion. Intraperitoneal injection was administered with a 12.7 mm 26 G needle at time 0 min followed by blood samples at 0, 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 0, 5, 15, 30, 90, 180, 240, 360, 480, and 600 min. Plasma was obtained by immediate centrifugation of blood samples for 5 min at 2500 g.

Protein Binding

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 above. The jugular vein was cannulated to provide a site for blood removal. Blood was removed from the rats 48 h post P407 injection and plasma was obtained by centrifugation for 5 min at 2500 g. 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. Nifedipine was dissolved in methanol (100 μg ml^{-1}) to provide a solution resulting in a concentration < 0.015% (v/v) methanol in the plasma samples. The plasma samples were spiked with 1.5 μg ml^{-1} nifedipine prior to incubation 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 (f_{up}) was calculated from $f_{up} = (U_1 + W_1 + W_2 + W_3 + W_4 + W_5)/C_s$, where U_1, W_1, W_2, W_3, W_4 and W_5 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 number of washes needed to achieve complete recovery of nifedipine. After completion of washes the membranes were collected and assayed for nifedipine.

Analysis of Total Serum Cholesterol

Total plasma cholesterol levels were determined approximately 5 min prior to nifedipine administration, 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.

Assay of Nifedipine

Plasma nifedipine concentrations were measured by a previously reported high performance liquid chromatography method (6). Calibration curves were linear ($r^2 > 0.99$) from 5 to 2000 ng ml^{-1} using 50–150 μl of rat plasma. Intra and inter-day variability was less than 10%. Millenium Chromatography Software Version 1.1 (Waters, Mississauga, ON, Canada) was used to integrate and process the chromatography.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic 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 $AUMC_{0\text{-}\infty} = AUMC_{0\text{-last}} + [t_{last} * (C_{last}/\lambda)] + (C_{last}/\lambda^2)$, respectively, where C_{last} is the last measureable concentration and t_{last} is the time of the last measurable concentration. The elimination rate constant (λ) was estimated by linear regression using the last 3–6 concentration data points. Total body clearance (CL_{TB}), and ip ($CL/F_{(ip)}$) and po clearance ($CL/F_{(po)}$) were calculated as Dose/$AUC_{0\text{-}\infty}$. Volume of distribution at steady state (Vdss) was calculated as Vdss = (Dose/$AUC_{0\text{-}\infty}$)*($AUMC_{0\text{-}\infty}$ / $AUC_{0\text{-}\infty}$). Absolute bioavailability (F) was calculated using the ratio of AUC after po or ip doses over that of iv.

Independent t-test's were used to evaluate the significance of differences in all cases within each route of administration. The one-way ANOVA followed by the Duncan New Multiple

Range test was used to determine the differences in AUC between routes of administration. 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 ± standard deviation.

RESULTS

Nifedipine was absorbed and eliminated rapidly so that the last collected samples generally contained no detectable concentration. The $AUC_{(0\text{-last})}$ therefore, was considered as the total AUC.

P407-induced hyperlipidemia caused significant elevations of total plasma cholesterol (Fig. 1). This resulted in significant decreased CL_{TB} and consequently increased AUC of nifedipine (Table I). Significant positive correlations in the hyperlipidemic rats were found between nifedipine AUC and total plasma cholesterol in the iv and po dosed rats ($r = 0.92$, $p = 0.028$, $r = 0.90$, $p = 0.016$, respectively) but not following ip doses (Fig. 2). There was a significant prolongation of $t_{1/2}$ following iv and ip doses. Half-life was not measured after po doses due to excessive fluctuation in concentrations in the elimination phase. Vdss was not effected by hyperlipidemia. The route of administration did not significantly influence the observed effects of P407-induced hyperlipidemia.

No differences in 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%, 14.86 ± 1.33% and 1.87 ± 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. Total serum 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.

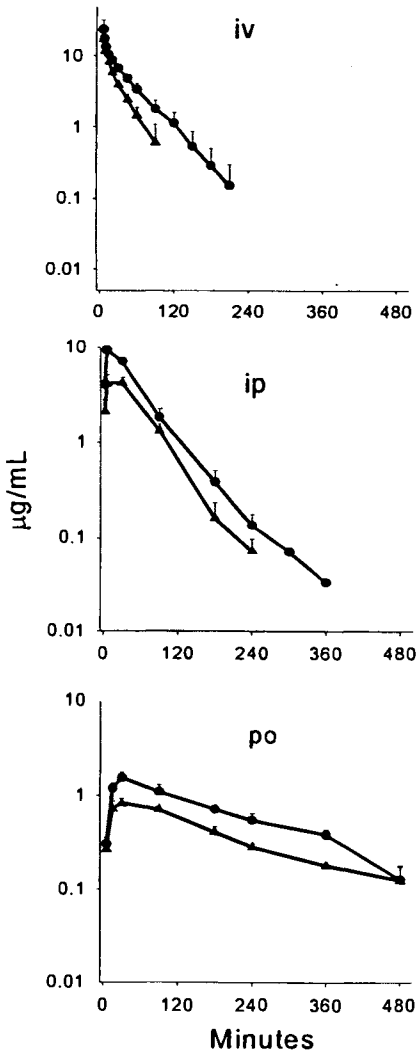

Fig. 1. Plasma nifedipine concentration-time curves following iv, ip, and po administration to normolipidemic ▲ and hyperlipidemic ● rats. Error bars represent standard deviation.

Table I. Comparison of Pharmacokinetic Data of Nifedipine in Normolipidemic and Hyperlipidemic Rats Administered via iv, ip, and po Routes (n = 5 or 6 Rats Per Group)

	iv		ip		po	
	Normal	High cholesterol	Normal	High cholesterol	Normal	High cholesterol
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-last} (ug min ml^{-1})	390 ± 49	639[b] ± 126	358 ± 59	638[b] ± 149	174[a] ± 46	297[b] ± 21
$AUC_{0-\infty}$ (ug min ml^{-1})	392 ± 49	647[b] ± 135	359 ± 58	640[b] ± 149	nd	nd
C_{max} (ug 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
Vdss (ml kg^{-1})	127 ± 34	113 ± 12				
CL_{TB} (ml min^{-1} kg^{-1})	4.5 ± 0.7	2.8[b] ± 0.5				
Cl/F (ml min^{-1} kg^{-1})			5.5 ± 1.1	3.0[b] ± 0.8	9.1 ± 0.8	5.3[b] ± 0.8
$t_{1/2}$ (min)	19 ± 4.0	31[b] ± 8.0	24 ± 5	36[b] ± 12	nd	nd
F	1.0	1.0	0.91	0.99	0.50	0.51

Note: Parameters are expressed as arithmetic means ± standard deviations.
[a] Significantly different from other routes of administration.
[b] Significantly different from normal. nd, Not determined.

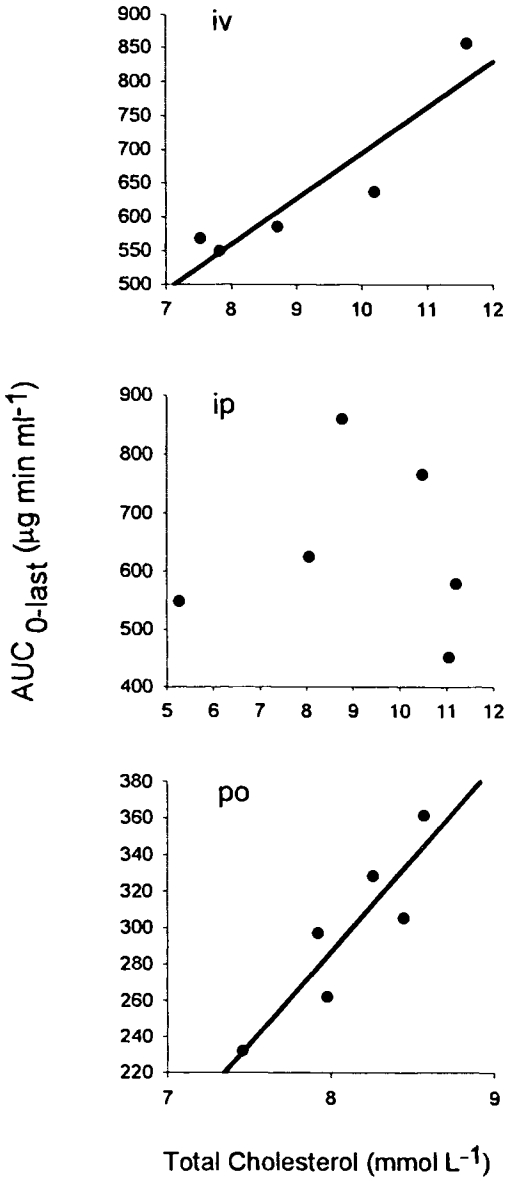

Fig. 2. Correlation of nifedipine AUC and total cholesterol concentration after iv ($r = 0.92$, $p < 0.05$), ip ($r = -0.16$, $p = 0.80$), and po ($r = 0.90$, $p < 0.05$) administration.

DISCUSSION

Nifedipine oral bioavailability in the rat (7) and human (8) is approximately 0.45–0.60 and 0.40–0.68, respectively. This low bioavailability has been attributed to a pre-systemic metabolism in the gut rather than hepatic first pass or incomplete absorption (7). Our observation that AUC of nifedipine following ip doses is almost equal to that observed after iv administration (Table I) further supports involvement of the gut rather than liver in the presystemic clearance of the drug.

Hyperlipidemia was induced in our rats using P407 that was adapted from a previously characterized model (5). Convenience and ability to replicate the human condition are two important issues when choosing an animal model. Although lipoprotein metabolism is substantially different than human, the rat is extensively used for lipid research. The plasma lipoprotein distribution that comprises total plasma cholesterol in the rat does not resemble that of human. Rats carry 60–80% of serum cholesterol in HDL, whereas humans carry 60–70% in LDL and only 20–30% in HDL (9). Perhaps an increase in protein binding resulting in a decrease in f_{up} occurs despite the differing lipoprotein distributions since nifedipine binds to both LDL and HDL (4). Furthermore, hyperlipidemia alters the pharmacokinetics of cyclosporine A similarly in the rat (10) and human (11).

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 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 (7).

It has been shown that lipoproteins interact with a number of lipophilic drugs and hyperlipidemia increases the binding capacity of the lipoproteins (2). In vitro studies have shown that nifedipine binds to albumin, AAG and lipoproteins with a f_{up} of 0.01–0.09 (4). Our data indicates that hyperlipidemia results in a significant 31% decrease in f_{up} of nifedipine in plasma (Table I). This is similar to previous reports suggestive of a negative correlation between lipoprotein concentrations and f_{up} for cyclosporine A (12), fentanyl (13), imipramine (14) and isradipine (15). In addition to CL_{TB}, Vdss 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 (Vt) or plasma volume (Vp) do not change. However, for drugs such as nifedipine that are extensively distributed, changes in Vdss are more likely dependent on tissue binding. The unchanged Vdss 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 Vdss.

Hyperlipidemia caused only a 31% reduction in f_{up} and approximately a 40% reduction in CL_{TB} regardless of the route of administration (Table I). Hence, the effect of hyperlipidemia on the pharmacokinetics of nifedipine may be 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 and/or a reduced intrinsic clearance cannot be ruled out.

In conclusion, P407-induce 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 and cytochrome P450 activity cannot be ruled out.

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