Pharmacokinetics and Pharmacodynamics of Nifedipine in Untreated and Atorvastatin-Treated Hyperlipidemic Rats

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Accepted for publication June 3, 1999
This paper is available online at http://www.jpet.org

ABSTRACT

Nifedipine, a hypertensive calcium channel blocker, is commonly administered to subjects with coronary heart disease who often exhibit hyperlipidemia. In general, the pharmacokinetic consequences of hyperlipidemia include increased total drug concentrations and decreased unbound fraction in plasma. However, the pharmacodynamic consequences of hyperlipidemia are conflicting; unaltered, increased, or decreased pharmacological effects are reported. In this study, the effect of experimental hyperlipidemia on pharmacokinetic and pharmacodynamic consequences of nifedipine was studied. After establishing a dose (0.05–0.3 mg kg\(^{-1}\))-effect relationship, single i.v. doses of nifedipine were administered to control and poloxamer 407-induced hyperlipidemic rats (with and without cholesterol-lowering agent atorvastatin) rats. Mean arterial pressure, total as well as unbound nifedipine plasma concentrations, and total cholesterol were monitored. Hyperlipidemia significantly decreased systemic clearance of nifedipine by 40% and increased \(T_{1/2}\) and area under the plasma concentration-time curve by 85 and 65%, respectively. Compared with the hyperlipidemic group, atorvastatin-treated rats had significantly lower total plasma cholesterol (0–70%), increased systemic clearance (39%), and decreased \(T_{1/2}\) (27%) and area under the plasma concentration-time curve (24%). Hyperlipidemia prolonged pharmacological \(T_{1/2}\) of nifedipine by 300%. Atorvastatin treatment significantly reduced this prolongation to 46%. There was a significant correlation between mean blood pressure and the total but not unbound nifedipine plasma concentrations. Hyperlipidemia potentiates the hypotensive effect of nifedipine by increasing its total plasma concentrations despite decreased unbound drug concentration.

Hyperlipidemia is defined as an elevation of one or more of the plasma lipids, including cholesterol, cholesteryl esters, triglycerides, or phospholipids (Raaesch, 1988). An elevation in plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs, or diseases (Raaesch, 1988). The pharmacokinetics (PK), particularly lipoprotein binding of drugs, may be altered in hyperlipidemic conditions, resulting in a decrease (Lindholm and Henricsson 1989) or increase (Wasan and Conklin 1997) in pharmacological response. Despite the differences in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic rat models are used extensively in lipid research. Many hyperlipidemic rat models exist. However, some have underlying pathological conditions such as hypothyroidism in the obese Zucker rat (Martin et al., 1978) and diabetes in the JCR-LA corpulent rat (Russell et al., 1989). Poloxamer 407 (P407), a nonionic surfactant, is nontoxic to cellular membranes and has successfully been used to induce hyperlipidemia in previous studies (Wout et al., 1992; Eliot et al., 1999). P407 was chosen as the hyperlipidemic model due to its convenience, reproducibility, and lack of undesirable underlying pathological conditions (Wout et al., 1992).

Cardiovascular diseases are the most common cause of death in most industrialized countries (Winocour, 1996). Hypertension and hyperlipidemia are two of the eight primary risk factors for coronary heart disease and their coexistence has been shown to be as high as 30% in many conditions (Winocour, 1996). Calcium channel blockers are currently one of the first-line therapeutic agents for the treatment of hypertension (Donnelly et al., 1994). In addition, the growing evidence of antiatherosclerotic properties of calcium channel blockers (Catapano, 1997) may make them an optimal choice of therapy for patients with coronary heart disease secondary to hypertension and hyperlipidemia. Many calcium channel blockers, particularly 1,4 dihydropyridines such as nifedipine (Rosenkranz et al., 1974), nicardipine (Urien et al., 1985), isradipine (Oravcova et al., 1994), and amlodipine (Oravcova et al., 1994) have been shown to bind to lipoproteins. However, the effect(s) of hyperlipidemia on the PK and pharmacodynamics (PD) of these drugs have not been investigated. The high prevalence of hyperlipidemia in subjects who require calcium channel blockers, coupled with the possibility...
of altered lipoprotein binding of the latter, calls for evaluation of the PK and PD of these drugs in hyperlipidemia. We report in this article, for the first time, the effects of hyperlipidemia and subsequent lowering of cholesterol using a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor, atorvastatin, on the PK and PD of nifedipine.

Nifedipine is highly bound to albumin, α1 acid glycoprotein, and to both low (LDL) and high-density lipoproteins (HDL) (Rosenkranz et al., 1974). Previous studies in our laboratory have shown that hyperlipidemia significantly decreases systemic clearance of nifedipine (Elliot et al., 1999); however, the effects of increased levels of total plasma cholesterol on the pharmacological response(s) of nifedipine have not been determined.

Materials and Methods

Chemicals and Dosage Forms. P407 (pluronic F127; BASF Corporation Canada Inc., Toronto, Ontario, Canada), 30% w/w solution, was made by dissolving the appropriate amount of P407 in cold saline and kept at 2–5°C to maintain a liquid phase 48 h before use. Tablets containing 40 mg of atorvastatin (Lipitor; Parke-Davis, Scarborough, Ontario, Canada) were crushed and suspended in 0.4% w/v methylcellulose to a final concentration of 30 mg/ml immediately before use. Nifedipine (Sigma Chemical Co., Mississauga, Ontario, Canada) was dissolved in polyethylene glycol (PEG 400; Union Carbide Chemicals, Danbury, CT) to a final concentration of 1 mg/ml the night before the PK-PD study to ensure complete dissolution.

Animals and Treatments. Experiments were performed on male Sprague-Dawley rats weighing between 340 and 400 g (Health Sciences Animal Services, University of Alberta, Edmonton, Canada). Procedures followed the ethics of animal investigation. Rats were kept in the study area 72 h before PK-PD experiments where they had access to food and water ad libitum. Intraperitoneal injection of pentobarbital sodium-induced anesthesia. For lowering total plasma cholesterol, 2 mg/kg atorvastatin (AT)-treated HL (HLA; n = 8) and 4 atorvastatin alone (n = 6). Baseline MAP and HR were taken in triplicate immediately after the completion of surgical procedures. No longer than 2 min later, nifedipine (0.1 mg/kg) was administered and its effect on MAP was recorded for 15 min.

Pharmacokinetic-Pharmacodynamic Study. The rats were grouped as follows: 1, control or NL (n = 7); 2, HL (n = 6); 3, atorvastatin (AT)-treated HL (HLA; n = 8); and 4, atorvastatin alone (n = 6). Baseline MAP and HR were taken in triplicate immediately after the completion of surgical procedures. No longer than 2 min later, nifedipine (0.1 mg/kg) was administered as a 30-s i.v. infusion. Blood samples of 150 to 250 μl were drawn at 0, 2, 5, 10, 15, 30, 45, 60, 90, and 120 min. Equal amounts of saline were administered to replace blood volume. After administration, the cannula was rinsed thoroughly with blood and saline. Plasma was obtained by immediate centrifugation of blood samples. To prevent nifedipine degradation, the blood and plasma samples were prepared, collected, and handled in a room illuminated only by sodium light. The samples were frozen at −20°C until analyzed. Pharmacodynamic measurements were taken from 2 to 50 s before each blood sampling and at 20 min. The carotid artery cannula was cleared of blood 15 s before measurement of blood pressure. The level of anesthesia throughout the study was monitored by the toe pinch (pedal) reflex. A maintenance dose of pentobarbital (20 mg/kg) was required at 60 to 75 min after the initial dose. Rats were kept warm by means of a wool blanket and heating lamp.

Noncompartmental analysis was used to describe the disposition of nifedipine. The elimination rate constant (λ) was estimated by linear regression with the last three to five concentration measurements of the time versus log concentration. The linear trapezoidal method and extrapolation to infinity was used to calculate area under the total concentration-time curve (AUC T0–∞) and area under the unbound concentration-time curve (AUC U0–∞). Total (AUC T0–∞) and unbound (AUC U0–∞) area under the concentration-time curve from 0 to 30 min also was calculated and used for interpretation of the PD data that were collected only for 30 min. Systemic clearance (Clsys) was calculated as Dose/AUC 0–30 min. Volume of distribution at steady state (Vdss) was calculated as Vdss = (Dose/kg)/AUC 0–∞, where AUMC 0–∞ is the area under the moment curve from the time of dosing to infinity. Cmax and tmax were read off the concentration-time profile. Area under the plasma concentration-time curve from 0 to 30 min (AUC 0–30 min) was calculated as AUC 0–30 min = (Dose/kg)/AUC 0–30 min. In NL and HL plasma, nifedipine t1/2 values were 0.035 ± 0.002 and 0.024 ± 0.001, respectively (Elliot et al., 1989). Noncompartmental analysis was used to calculate the AUC from 0 to 30 min. The effect curve of percentage decrease in MAP, where E is the effect (percentage of decrease in MAP), Emax is the maximum reduction in MAP, C is the nifedipine plasma concentration, EC50 is the concentration at 50% of maximum effect, and n is the Hill coefficient. Percentage of change in MAP and HR versus time curves were constructed for each dose.

Anesthetic Study. The effects of pentobarbital sodium-induced anesthesia on MAP and HR were determined in normolipidemic (NL) (n = 3) and hyperlipidemic (HL; n = 3) rats. Following the surgical procedures and preparations that required −30 min, the effects of the initial dose (65 mg/kg) at 30 to 60 min following administration were studied. Subsequently, a maintenance dose (20 mg/kg) was administered, and its effect on MAP was recorded for 15 min.

Pharmacokinetic-Pharmacodynamic Study. The rats were grouped as follows: 1, control or NL (n = 7); 2, HL (n = 6); 3, atorvastatin (AT)-treated HL (HLA; n = 8); and 4, atorvastatin alone (n = 6). Baseline MAP and HR were taken in triplicate immediately after the completion of surgical procedures. No longer than 2 min later, nifedipine (0.1 mg/kg) was administered as a 30-s i.v. infusion. Blood samples of 150 to 250 μl were drawn at 0, 2, 5, 10, 15, 30, 45, 60, 90, and 120 min. Equal amounts of saline were administered to replace blood volume. After administration, the cannula was rinsed thoroughly with blood and saline. Plasma was obtained by immediate centrifugation of blood samples. To prevent nifedipine degradation, the blood and plasma samples were prepared, collected, and handled in a room illuminated only by sodium light. The samples were frozen at −20°C until analyzed. Pharmacodynamic measurements were taken from −5 to 30 s before each blood sampling and at 20 min. The carotid artery cannula was cleared of blood 15 s before measurement of blood pressure. The level of anesthesia throughout the study was monitored by the toe pinch (pedal) reflex. A maintenance dose of pentobarbital (20 mg/kg) was required at 60 to 75 min after the initial dose. Rats were kept warm by means of a wool blanket and heating lamp.

Noncompartmental analysis was used to describe the disposition of nifedipine. The elimination rate constant (λ) was estimated by linear regression with the last three to five concentration measurements of the time versus log concentration. The linear trapezoidal method and extrapolation to infinity was used to calculate area under the total concentration-time curve (AUC T0–∞) and area under the unbound concentration-time curve (AUC U0–∞). Total (AUC T0–∞) and unbound (AUC U0–∞) area under the concentration-time curve from 0 to 30 min also was calculated and used for interpretation of the PD data that were collected only for 30 min. Systemic clearance (Clsys) was calculated as Dose/AUC 0–30 min. Volume of distribution at steady state (Vdss) was calculated as Vdss = (Dose/kg)/AUC 0–∞, where AUMC 0–∞ is the area under the moment curve from the time of dosing to infinity. Cmax and tmax were read off the concentration-time profile. Area under the plasma concentration-time curve from 0 to 30 min (AUC 0–30 min) was calculated as AUC 0–30 min = (Dose/kg)/AUC 0–30 min. In NL and HL plasma, nifedipine t1/2 values were 0.035 ± 0.002 and 0.024 ± 0.001, respectively (Elliot et al., 1989). Noncompartmental analysis was used to calculate the AUC from 0 to 30 min. The effect curve of percentage decrease in MAP, where E is the effect (percentage of decrease in MAP), Emax is the maximum reduction in MAP, C is the nifedipine plasma concentration, EC50 is the concentration at 50% of maximum effect, and n is the Hill coefficient. Percentage of change in MAP and HR versus time curves were constructed for each dose.
HR was taken as the length of the R-R interval of the lead I electrocardiogram. The pressure transducer was calibrated daily. Calibration curves from 30 to 150 mm Hg were linear, $r^2 = 0.99$, and intra- and interday variability was <10%.

Pharmacodynamic analysis included measurements of maximum percentage of decrease ($E_{\max}$) of MAP, area under the effect-time curves from time 0 to 30 min (AUEC$_{0–30}$ min), and pharmacological $T_{1/2}$. Maximum percentage of decrease was interpreted from the effect-time curves, all of which occurred at 2 min.

The percentage of decrease in MAP was related to the nifedipine plasma concentration by means of the linear model (Venitz, 1995) according to $E = m \times C + b$, where $E$ is the measured effect (percentage of decrease in MAP), $C$ is nifedipine plasma concentration in ng ml$^{-1}$, $m$ is the slope, and $b$ is the $y$-intercept.

**Statistical Analysis.** Statistical comparisons for the pharmacokinetic-pharmacodynamic study among the NL, HL, HLA, and AT groups and dose-response study among doses 0.05, 0.1, and 0.3 mg kg$^{-1}$ were carried out with a one-way ANOVA with Fischer's least-significant difference test for multiple comparisons. Comparisons between the NL and HL rats in the pentobarbital sodium-induced anesthesia study were made by independent $t$ tests. PK parameters, except $T_{\max}$, were log transformed before statistical analysis. A $p \leq .05$ was considered to be statistically significant. Data are reported as mean ± S.D. Linear regressions (Sigma Plot; SPSS Inc., Chicago, IL) were used to determine the relationship between total plasma cholesterol and pharmacokinetic and pharmacodynamic parameters.

**Analysis of Total Plasma Cholesterol.** Total plasma cholesterol was determined with blood samples that were drawn 2 min before the study. The Surgical Medical Research Institute (University of Alberta) performed the analytical tests. A colorimetric assay with dry chemistry technology was used and analyzed in a VETTEST 8008 (Innex Canada Corporation, Toronto, Ontario, Canada).

**Analysis of Nifedipine.** Plasma nifedipine concentrations were measured by a previously reported HPLC method (Grundy et al., 1994). The method involved extraction with methyl-$t$-butyl ether:iso-octane (75:25 v/v) and subsequent evaporation of the resulting organic phase. The residue was reconstituted with a 200-$\mu$L mobile phase (methanol:water:acetic acid, 65:34:1 v/v plus an additional 300 $\mu$L of triethylamine). Nifedipine was detected by UV/visible at 350 nm. Calibration curves were linear ($r^2 > 0.999$) from 5 to 2000 ng ml$^{-1}$. Intra- and interday variability was <8%.

**Results**

Thirty minutes after the administration of anesthesia, the surgical procedures were completed. Baseline MAP and HR were similar in the NL and HL groups. MAP gradually increased from baseline, at 30 min by 0.2% in NL and by 1.5% in HL at 32 min, to ~8% at 60 min in both groups. Throughout this study period, no significant differences in MAP and HR between the NL and HL groups were detected, ruling out any significant differences in hemodynamic alterations by 65 mg kg$^{-1}$ pentobarbital sodium between the NL and HL groups. Unlike MAP, HR displayed no consistent change throughout the duration of the study with a substantial degree of inter- and intrasubject variability. Maintenance doses of pentobarbital (20 mg kg$^{-1}$) were required at 30 min as determined by a positive response to the pedal reflex. Fifteen minutes after the maintenance dose, MAP decreased by 9 ± 2% and 6 ± 5% in NL and HL groups, respectively.

Fig. 1 depicts the concentration-dependent and -independent reduction in MAP and HR, respectively. Therefore, MAP, but not HR, was used as the pharmacological endpoint of nifedipine. The percentage of reduction in MAP was described by the sigmoidal $E_{\max}$ model, $E_{\max}$, $EC_{50}$, 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.

Total plasma cholesterol concentrations were 1.4 ± 0.3 and 1.4 ± 0.2 mmol l$^{-1}$ in the NL and AT groups, respectively. P407 significantly increased total cholesterol by 8.9-fold (12.4 ± 1.1 mmol l$^{-1}$). Administration of atorvastatin to the HLA group decreased total cholesterol by 0 to 70% (9.2 ± 3.5 mmol l$^{-1}$), which was significantly lower than that of the HL group but still significantly higher than the NL and atorvastatin groups.

AUC $T_{\text{0}–\infty}$ was significantly increased by 66% in the HL group compared with the NL and AT groups (Table 1). Atorvastatin treatment (HLA group) decreased AUC $T_{\text{0}–\infty}$ to a level not significantly different from those of the NL and AT groups. $Cl_{\text{sys}}$ of nifedipine was affected to a similar extent as AUC $T_{\text{0}–\infty}$. A significant negative correlation ($r = 0.67$, $p < .004$) between $Cl_{\text{sys}}$ of nifedipine and total plasma cholesterol was found in hyperlipidemic rats (Fig. 2). Consequently, a positive correlation between AUC $T_{\text{0}–\infty}$ and total plasma cholesterol levels was found ($r = 0.71$, $p < .0001$). Plasma nifedipine concentration-time curves were superimposable in all.
TABLE 1
Pharmacokinetic parameters of nifedipine (0.1 mg·kg⁻¹ i.v.) in NL, HL, HLA, and AT groups

<table>
<thead>
<tr>
<th></th>
<th>NL</th>
<th>AT</th>
<th>HLA</th>
<th>HL</th>
<th>Statistical Comparisons*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCU₀-&gt;∞ (µg·ml⁻¹ min)</td>
<td>17.2 ± 2.6</td>
<td>17.9 ± 6.6</td>
<td>21.7 ± 5.8</td>
<td>28.5 ± 3.3</td>
<td>AT NL HLA HL</td>
</tr>
<tr>
<td>AUCU₀-&gt;∞ (µg·ml⁻¹ min)</td>
<td>0.6 ± 0.09</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.7 ± 0.08</td>
<td>NL HL</td>
</tr>
<tr>
<td>AUCU₀–30 min (µg·ml⁻¹ min)</td>
<td>8.3 ± 0.8</td>
<td>n.d.</td>
<td>n.d.</td>
<td>9.0 ± 1.2</td>
<td>NL HL</td>
</tr>
<tr>
<td>AUCU₀–30 min (µg·ml⁻¹ min)</td>
<td>0.3 ± 0.03</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.2 ± 0.02</td>
<td>NL HL</td>
</tr>
<tr>
<td>CLsys (ml·min⁻¹·kg⁻¹)</td>
<td>5.92 ± 0.93</td>
<td>6.14 ± 1.73</td>
<td>4.95 ± 1.54</td>
<td>3.55 ± 0.42</td>
<td>AT NL HLA HL</td>
</tr>
<tr>
<td>T₁/₂ (min)</td>
<td>34 ± 11</td>
<td>30 ± 12</td>
<td>46 ± 15</td>
<td>63 ± 14</td>
<td>AT NL HLA HL</td>
</tr>
<tr>
<td>Vdss (ml·kg⁻¹)</td>
<td>268 ± 44</td>
<td>227 ± 22</td>
<td>278 ± 35</td>
<td>309 ± 34</td>
<td>AT NL HLA HL</td>
</tr>
</tbody>
</table>

* n.d., not determined. Groups connected with underline are not significantly different. Data are presented as mean ± S.D.

**Fig. 2.** Correlation (r = 0.70, p < .028) between nifedipine systemic clearance and total plasma cholesterol concentration in hyperlipidemic rats (HL and HLA). Solid line represent the best fit. For NL rats, no significant correlation was found between the two parameters.

**Fig. 3.** Nifedipine concentration and effect versus time curves following i.v. administration of 0.1 mg·kg⁻¹ nifedipine. NL (●, n = 5), HL (■, n = 6), HLA (▲, n = 8), and AT (♦, n = 6) groups. Top panel, significant differences (p < .05) between HL and NL (a) and between HL and atorvastatin (b) groups. Bottom panel, significant differences (p < .05) between HL and all other groups only at 30 min. Data are presented as mean ± S.E.

Discussion

Nifedipine is a potent arterial vasodilator that decreases systemic vascular resistance and blood pressure. Unfortunately,
sympathetic stimulation, due to a reflex baroreceptor mechanism, may result in tachycardia. Studies in unconscious rats have found that nifedipine induces a concentration-dependent and -independent decrease in MAP and HR, respectively (Achike and Dai, 1990). As shown in Fig. 1, a sigmoidal nifedipine plasma concentration-effect relationship within the examined dosage range (0.05–0.30 mg kg⁻¹) was observed. The chosen dose of 0.1 mg kg⁻¹ was within the log-linear phase of the curve. HR was not used as a pharmacological endpoint due to an excessive variability in response (Fig. 1).

Nifedipine is metabolized to inactive metabolites in the gastrointestinal tract and liver by cytochrome P-450 3A in the human and an additional 2C subclass in the rat (Dokladalova et al., 1982; Guengerich et al., 1986). As found in this study, previous work in our laboratory showed a significant reduction in Clsys (40%) and increases in AUC as measured by AUEC₀–₃₀ min and slope of the linear-effect curves (Table 2). Indeed, an increasing trend in effect, which amounted to a significantly higher effect, at 30 min was observed. This effect was accompanied by a significant prolongation of both τ₁/₂ of the concentration-time curve and pharmacological τ₁/₂ and a significant correlation between the two indices. A lack of a corresponding decrease in the unbound nifedipine concentration (AUC U₀–₃₀ min) and pharmacological effect (AUC₀–₃₀ min) may suggest that the pharmacological activity (lowering of MAP) of nifedipine is dependent on total rather than unbound nifedipine concentrations. This interesting observation is contrary to the general belief that usually only the unbound fraction of the circulating drug is pharmacologically active. However, previous observations (Brajtburg et al., 1984; Danon and Chen, 1998) that hyperlipidemia does not impair drug activity, but rather, may facilitate drug uptake into tissue (Gurusinge et al., 1988) support our observation. The site of action of nifedipine is in the vascular smooth muscle and although the exact mechanism of binding has not been determined, it has been suggested that nifedipine may reach its receptor via plasma lipids (Ferrari, 1997). This explanation is plausible because lipoproteins interact with vascular smooth muscle, whereby LDL brings cholesterol to the smooth muscle and HDL removes it (Fielding and Fielding, 1996), as evidenced by the role of LDL and HDL in atherosclerosis and reverse cholesterol transport, respectively. Perhaps nifedipine transfer is augmented by plasma lipid transfer to the smooth muscle tissue. Our observation suggests that the therapeutic outcome of an increased binding to lipoproteins may be different from that of plasma proteins such as albumin and α₁ acid glycoproteins.

Although we collected blood samples for the pharmacokinetic experiment for 120 min, the pharmacological effect of nifedipine was followed for only 30 min because the effect in the NL rats did not last >30 min. Also, the maintenance doses of pentobarbital sodium that were required at 30 min tended to decrease MAP. During the 30-min blood pressure monitoring, AUC of unbound nifedipine was significantly lower in HL compared with NL rats (Table 1). This, however, did not result in a decrease in pharmacological effect as measured by AUEC₀–₃₀ min and slope of the linear-effect curves (Table 2). Indeed, an increasing trend in effect, which amounted to a significantly higher effect, at 30 min was observed. This was accompanied by a significant prolongation of both τ₁/₂ of the concentration-time curve and pharmacological τ₁/₂ and a significant correlation between the two indices. A lack of a corresponding decrease in the unbound nifedipine concentration (AUC U₀–₃₀ min) and pharmacological effect (AUEC₀–₃₀ min) may suggest that the pharmacological activity (lowering of MAP) of nifedipine is dependent on total rather than unbound nifedipine concentrations. This interesting observation is contrary to the general belief that usually only the unbound fraction of the circulating drug is pharmacologically active. However, previous observations (Brajtburg et al., 1984; Danon and Chen, 1998) that hyperlipidemia does not impair drug activity, but rather, may facilitate drug uptake into tissue (Gurusinge et al., 1988) support our observation. The site of action of nifedipine is in the vascular smooth muscle and although the exact mechanism of binding has not been determined, it has been suggested that nifedipine may reach its receptor via plasma lipids (Ferrari, 1997). This explanation is plausible because lipoproteins interact with vascular smooth muscle, whereby LDL brings cholesterol to the smooth muscle and HDL removes it (Fielding and Fielding, 1996), as evidenced by the role of LDL and HDL in atherosclerosis and reverse cholesterol transport, respectively. Perhaps nifedipine transfer is augmented by plasma lipid transfer to the smooth muscle tissue. Our observation suggests that the therapeutic outcome of an increased binding to lipoproteins may be different from that of plasma proteins such as albumin and α₁ acid glycoproteins.

Both the HL and HLA groups tend to exhibit somewhat larger VDₘₐ than the NL and AT groups (Table 1). These numerical differences resulted in statistical significance only between the AT group and the high cholesterol groups (HL and HLA). This finding may further support the notion of nifedipine distribution by plasma lipids. The NL group showed the greatest variability in VDₘₐ, which may explain its lack of significant differences with the other groups.

Reduction of total plasma cholesterol levels with atorvastatin intervention (HLA group) increased Clsys and shortened τ₁/₂ of nifedipine to levels that were not significantly different from

### Table 2: Pharmacodynamic indices calculated with MAP after i.v. administration of 0.1 mg · kg⁻¹ nifedipine in NL, HL, HLA, or AT group

<table>
<thead>
<tr>
<th></th>
<th>NL</th>
<th>AT</th>
<th>HLA</th>
<th>HL</th>
<th>Statistical Comparisons*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUEC₀–₃₀ min (mm Hg · min)</strong></td>
<td>407.8 ± 90.0</td>
<td>425.9 ± 175.3</td>
<td>372.0 ± 101.3</td>
<td>477.7 ± 93.1</td>
<td>AT NL HLA HL</td>
</tr>
<tr>
<td><strong>Max % decrease in MAP</strong></td>
<td>37 ± 6</td>
<td>37 ± 2</td>
<td>32 ± 2</td>
<td>33 ± 5</td>
<td>AT NL HLA HL</td>
</tr>
<tr>
<td><strong>Pharmacological τ₁/₂ (min)</strong></td>
<td>7 ± 2</td>
<td>7 ± 3</td>
<td>15 ± 8</td>
<td>28 ± 10</td>
<td>AT NL HLA HL</td>
</tr>
<tr>
<td><strong>Slope (m) of linear effect-concentration profile</strong></td>
<td>0.097 ± 0.017</td>
<td>0.088 ± 0.026</td>
<td>0.084 ± 0.021</td>
<td>0.080 ± 0.036</td>
<td>AT NL HLA HL</td>
</tr>
</tbody>
</table>

*Groups connected with underline are not significantly different. Data are presented as mean ± S.D.

![Fig. 4. Total concentration-effect relationship (p < .05) after 0.1 mg kg⁻¹ i.v. dose of nifedipine in NL (●, r² = 0.997, m = 0.097), HL (■, r² = 0.970, m = 0.075), HLA (▲, r² = 0.987, m = 0.084), and AT (◆, r² = 0.990, m = 0.088) groups. Error bars represent S.E.M.](image-url)
those of the NL group. Reduction of total plasma cholesterol in the HLA group significantly decreased pharmacological $T_{\text{H}}$ of nifedipine compared with that of the HL group. However, pharmacological $T_{\text{H}}$ remained significantly higher in the HLA group compared with the NL group. These findings are similar to previous studies in humans using simvastatin in hyperlipidemic renal transplant recipients, where cyclosporine A trough concentrations decreased and unbound increased in simvastatin-treated recipients compared with untreated recipients (Akhlaghi et al., 1997). In addition, the significant positive correlations between drug concentrations and total plasma cholesterol levels observed in this study (Fig. 2) are similar to previous findings for cyclosporine A (Lithell et al., 1986; Wasan and Conklin, 1997). In addition, the observed significant negative correlations between total plasma cholesterol and nifedipine Clsys found for nifedipine are comparable to those reported for cyclosporine A (Lithell et al., 1986).

Extrapolation of these results from rat to human is plausible given the similarities in alterations of cyclosporine A concentrations in plasma (Lithell et al., 1986; Brunner et al., 1988) and amphoterin B toxicities (Vadiee et al., 1990; Wasan and Conklin, 1997) in the hyperlipidemic rat and human.

Nifedipine has been suggested to be associated with an increased mortality and incidence of cardiovascular events in patients with established coronary heart disease (Furberg et al., 1995). In addition, nifedipine-induced reflex tachycardia, leading to a loss of blood pressure control, has been positively correlated to plasma concentrations. Perhaps future studies investigating the effects of hyperlipidemia on nifedipine PK and PD in humans may help resolve the ongoing controversy of the increased risk associated with nifedipine in coronary heart disease (Eliot, 1998), a condition associated with hyperlipidemia.

**Conclusions**

Hyperlipidemia decreased Clsys of nifedipine and increased $T_{\text{H}}$ with a nonsignificant increasing trend in $V_{\text{d,sys}}$. Lowering of MAP was sustained and the pharmacological $T_{\text{H}}$ was prolonged in the HL state, which corresponded with increases in total plasma nifedipine concentrations. Atorvastatin reduced P407-induced hyperlipidemia and brought pharmacokinetic parameters back to the normal range. In addition, atorvastatin treatment partially normalized the lowering effect of nifedipine on blood pressure. The effect of nifedipine on MAP seems to correlate better with total rather than unbound plasma concentrations. Thus, high total plasma nifedipine concentrations in the HL state may alter its therapeutic outcome.

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