

Effects of Atorvastatin on the Pharmacokinetics of Nicardipine after Oral and Intravenous Administration in Rats

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Abstract – The aim of this study was to investigate the effect of atorvastatin on the pharmacokinetics of nicardipine after oral and intravenous administration of nicardipine to rats. Nicardipine was administered orally (12 mg/kg) or intravenously (i.v., 4 mg/kg) without or with oral administration of atorvastatin (0.3 or 1.0 mg/kg) to rats. The effect of atorvastatin on the P-glycoprotein (P-gp) as well as CYP3A4 activity was also evaluated. Atorvastatin inhibited CYP3A4 enzyme activity in a concentration-dependent manner with 50% inhibition concentration (IC₅₀) of 48 μM. Compared to the controls (nicardipine alone), the area under the plasma concentration-time curve (AUC) of nicardipine was significantly (1.0 mg/kg, $p < 0.05$) greater by 16.8-45.4%, and the peak plasma concentration (C_{max}) was significantly (1.0 mg/kg, $p < 0.05$) higher by 28.0% after oral administration of nicardipine with atorvastatin, respectively. Consequently, the relative bioavailability (R.B.) of nicardipine was increased by 1.17- to 1.45-fold and the absolute bioavailability (A.B.) of nicardipine with atorvastatin was significantly greater by 16.7-20.9% compared to that of the controls (14.3%). Compared to the i.v. control, atorvastatin did not significantly change pharmacokinetic parameters of i.v. administration nicardipine. The enhanced oral bioavailability of nicardipine by atorvastatin suggests that CYP3A subfamily-mediated metabolism were inhibited in the intestine and/or in the liver rather than P-gp-mediated efflux of nicardipine. Based on these results, modification of nicardipine of dosage regimen is required in the patients. Human studies are required to prove the above hypothesis.

Keywords: Nicardipine, Atorvastatin, Bioavailability, Pharmacokinetics, CYP3A subfamily, P-gp, Rats

INTRODUCTION

Nicardipine, a dihydropyridine calcium channel antagonist, causes coronary and peripheral vasodilatation by blocking the influx of extracellular calcium across cell membranes. Nicardipine is arterioselective and effective for the treatment of hypertension, myocardial ischemia, and vasospasm in surgical patients (Kishi *et al.*, 1984; Hysing *et al.*, 1986). Nicardipine has also been used experimentally as a probe to study the effects of calcium channel antagonists on the role of sympathetic nervous system activity in the development of cardiovascular risk (Van Swieten *et al.*, 1997). The pharmacokinetic parameters of nicardipine are non-linear due to hepatic first-pass metabolism, thus, the extent of oral bioavailability (F) was

low about 35% following a 30 mg dose at steady state (Graham *et al.*, 1984; Graham *et al.*, 1985). It is a substrate of cytochrome P450 (CYP) 3A subfamily, especially CYP3A4 in humans and forms to pharmacologically inactive metabolite (Higuchi and Shiobara, 1980; Guengerich *et al.*, 1986; Guengerich, 1991). In addition, nicardipine is also a P-glycoprotein (P-gp) substrate (Hu *et al.*, 1996; Wang *et al.*, 2000).

Atorvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, is widely used to prevent the progression of atherosclerosis by lowering plasma low-density lipid (LDL) levels in patients with hypercholesterolemia (Doppenschmitt *et al.*, 1999). Atorvastatin has been identified as a substrate of CYP3A4 which could be controlling factors for the low systemic availability of atorvastatin (Lea and McTavish, 1997). Accordingly, previous studies have revealed that many CYP3A4 inhibitors including itraconazole, clarithromycin, azithromycin, nelfinavir

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and grapefruit juice increased the bioavailability of atorvastatin (Lennernas, 2003). Holtzman *et al.* (2006) reported that HMG-CoA reductase inhibitors (statins) are inhibitors of P-gp in the rodent system, but the effects of atorvastatin on the inhibition of CYP3A4 and P-gp activity are partially ambiguous. Thus we attempted to re-evaluate CYP3A4 and P-gp activity using rhodamine-123 retention assay in P-gp-overexpressed MCF-7/ADR cells. This may be explained in part by the difference in the transport mechanisms of hydrophilic and lipophilic statins. For atorvastatin, a hydrophilic statin, specific transporter(s) including OATP1B1 are involved in its hepatic uptake (Shitara and Sugiyama, 2006) and thus the change of the metabolic rate itself may not drastically alter the pharmacokinetics of atorvastatin while it makes a greater alteration in those of simvastatin and lovastatin whose membrane transports are mainly mediated by passive diffusion (Lennernas, 2003). Antihypertensive agents are commonly co-administered with cholesterol-lowering agents in clinics. There are some reports on the effects of calcium channel antagonists on the pharmacokinetics of HMG-CoA reductase inhibitors. Calcium-channel blockers increased plasma concentrations of some statins (lovastatin, pravastatin and simvastatin), possibly through the inhibition of CYP 3A4 and P-gp (Azie *et al.*, 1998; Mousa *et al.*, 2000). But there are fewer reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats (Choi *et al.*, 2006; Choi *et al.*, 2008). Moreover, atorvastatin and nicardipine could be prescribed for the prevention or treatment of cardiovascular diseases as a combination therapy.

The low bioavailability of oral nicardipine is mainly due to pre-systemic metabolism by CYP3A4 and P-gp mediated efflux in the intestine. Therefore, the present study aimed to investigate the effect of atorvastatin on the intravenous and oral pharmacokinetics of nicardipine in rats.

MATERIALS AND METHODS

Chemicals and apparatus

Nicardipine, atorvastatin and nimodipine [internal standard for high-performance liquid chromatograph (HPLC) analysis of nicardipine] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade acetonitrile was a product from Merck Co. (Darmstadt, Germany). Other chemicals for this study were of reagent grade.

HPLC system used in this study were a Waters 1515 isocratic HPLC pump, a Waters 717 plus autosampler and a WatersTM 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a HPLC column temperature con-

troller (Phenomenex Inc., CA, USA), a Bransonic[®] Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA) and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague-Dawley rats of 7-8 weeks of age (weighing 270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Co., Gangwon, Republic of Korea) and tap water *ad libitum*. The animals were housed (two rats per cage) in a clean-room maintained at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of 50-60%, with 12-h light and dark cycles. The rats were acclimated under these conditions for at least 1 week. All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) and the Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the protocol of this animal study. The rats were fasted for at least 24-h prior to beginning the experiments and had free access to tap water. Each animal was anaesthetized with light ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and drug administration, respectively.

Oral and intravenous administration of nicardipine

The rats were randomly divided into four groups ($n=6$, each); an oral group (12 mg/kg of nicardipine dissolved in water; homogenized at 36°C for 30 min; 3.0 ml/kg) without (control) or with 0.3 or 1.0 mg/kg of oral atorvastatin, and an i.v. group (4 mg/kg of nicardipine, dissolved in 0.9% NaCl-injectable solution; homogenized at 36°C for 30 min; 1.5 ml/kg) without (control) or with 0.3 or 1.0 mg/kg of oral atorvastatin. Nicardipine was administered orally using a gastric gavage tube, and atorvastatin was orally administered 30 min prior to oral or intravenous administration of nicardipine. Nicardipine for i.v. administration was injected through the femoral vein within 0.5 min. A blood sample (0.45 ml) was collected into heparinized tubes from the femoral artery at 0 (control), 0.017 (end of the infusion), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after intravenous infusion, and 0.1, 0.25, 0.5, 1, 2, 3, 6, 8, 12 and 24 h after oral administration. The blood samples were centrifuged (13,000 rpm, 5 min), and the plasma samples were stored at -40°C until HPLC analysis of nicardipine. An approximately 1 ml of whole blood collected from un-

treated rats was infused via the femoral artery at 0.25, 1, 3 and 8 h, respectively, to replace the blood loss due to blood sampling.

HPLC assay

The plasma concentrations of nicardipine were determined by a HPLC assay method reported by Eastwood *et al.* (1990). Briefly, a 50- μ l aliquot of nimodipine (2 μ g/ml), a 20- μ l aliquot of 2 N sodium hydroxide solution and 1.2-ml of tert-butylmethylether:hexane (75:25) were added to 0.2-ml aliquot of plasma sample. The mixture was then stirred for 2 min and centrifuged (13,000 rpm, 10 min). A 1.0 ml aliquot of the organic layer was transferred to a clean test tube and evaporated under a gentle stream of nitrogen at 35°C. The residue was dissolved in 200- μ l of the mobile phase and centrifuged (13,000 rpm, 5 min). A 50- μ l aliquot of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a Symmetry[®] C₁₈ column (4.6 \times 150 mm, 5 μ m, Waters), and a μ Bondapak[™] C₁₈ HPLC Precolumn (10 μ m, Waters). The mobile phase was acetonitrile:0.015 M KH₂PO₄ (60:40, v/v, pH 4.5) with 2.8 mM triethylamine, which was run at a flow rate of 1.5 ml/min. Chromatography was performed at a temperature of 30°C that was set by a HPLC column temperature controller. The UV detector was set to 254 nm. The retention times of nicardipine and the internal standard were 7.8 and 4.2 min, respectively. The detection limit of nicardipine in rat's plasma was 5 ng/ml. The coefficients of variation for nicardipine were below 14.1%.

CYP inhibition assay

The inhibition assays of human CYP3A4 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (BD Bioscience, San Jose, CA). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP3A4 substrate (7-Benzyloxy-4-(trifluoromethyl)couamrin (BFC)) was incubated with or without test compounds in a reaction mixture containing 1 pmol of CYP3A4 enzyme and the NADPH-generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM MgCl₂) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45 min. Metabolite concentrations were measured with a spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μ M ketoconazole) was run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and the re-

sults are expressed as the percent of inhibition.

Rhodamine-123 retention assay

The P-gp-overexpressed multidrug resistant human breast carcinoma cell line (MCF-7/ADR cells) was seeded in 24-well plates. At 80% confluence, the cells were incubated in fetal bovine serum (FBS)-free Dulbecco's modified Eagle's medium (DMEM) for 18 h. The culture medium was changed with Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20- μ M rhodamine-123 in the presence or absence of atorvastatin (1, 3 or 10 μ M) or verapamil (100 μ M) for 90 min, the medium was completely aspirated. The cells were then washed three times with an ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the percentage ratio to control.

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using a non-compartmental analysis (WinNonlin; software version 4.1; Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of nicardipine concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}) of nicardipine were directly read from the experimental data. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el} . Total body clearance (CL) was calculated by Dose/AUC. The absolute bioavailability (F.%) of nicardipine was calculated by $AUC_{oral}/AUC_{iv} \times Dose_{i.v.}/Dose_{oral} \times 100$, and the relative bioavailability (R.B.%) of nicardipine was estimated by $AUC_{with\ atorvastatin}/AUC_{control} \times 100$.

Statistical analysis

All data are expressed with their standard deviation (mean \pm SD). Statistical analysis was conducted using a one-way analyses of variance (ANOVA) followed by a *posteriori* testing with Dunnett's correction. Differences were considered significant at a level of $p < 0.05$.

RESULTS

Inhibitory effect of atorvastatin on CYP3A4

The inhibitory effect of atorvastatin on CYP3A4 activity is shown in Fig. 1. Atorvastatin inhibited CYP3A4 enzyme activity in a concentration-dependent manner and the 50 % inhibition concentration (IC_{50}) values of atorvastatin on

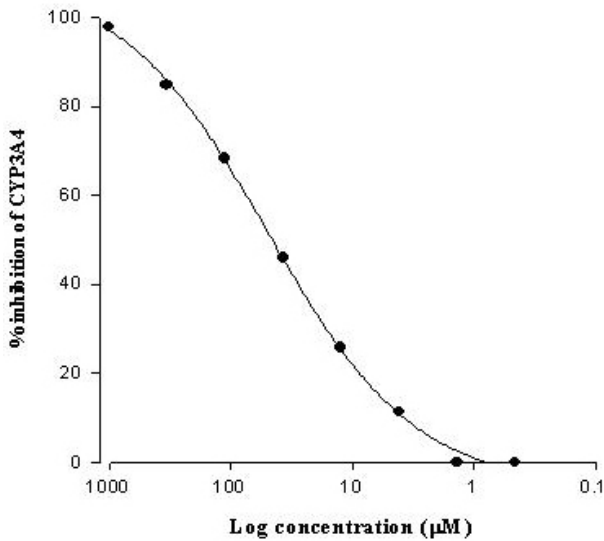


Fig. 1. Inhibitory effect of atorvastatin on CYP3A4 activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

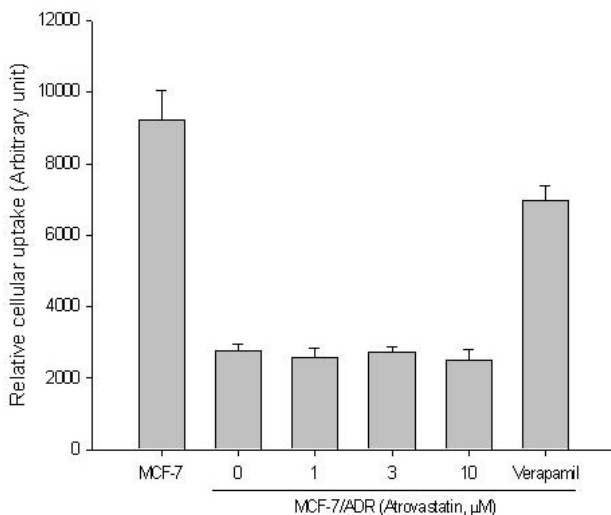


Fig. 2. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with atorvastatin for 30 min. After incubation of MCF-7/ADR cells with 20 μ M R-123 for 90 min. The values were divided by total protein contents of each sample. Verapamil (100 μ M) was used as a positive control. Data represents mean \pm SD of 6 separate samples.

CYP3A4 activity was determined as 48 μ M.

Rhodamine-123 retention assay.

As shown in Fig. 2, accumulation of rhodamine-123, a P-gp substrate, was not reduced in MCF-7/ADR cells over-expressing P-gp compared to that in MCF-7 cells lacking

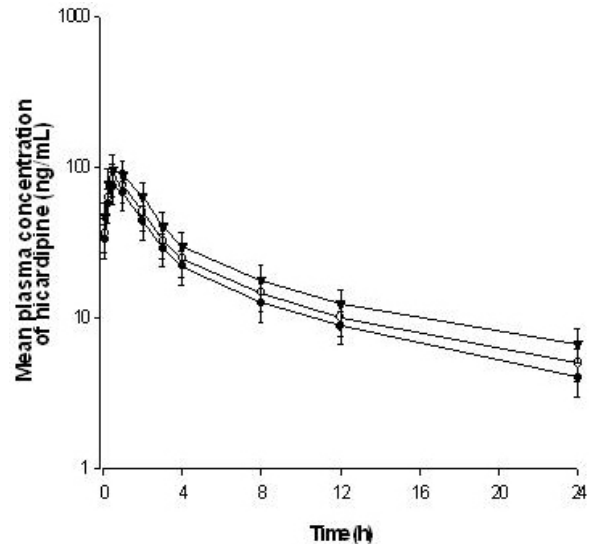


Fig. 3. Mean arterial plasma concentration-time profiles of nicardipine after its oral administration (12 mg/kg) without (●) or with 0.3 mg/kg (○) and 1.0 mg/kg (▼) of atorvastatin to rats. Bars represent the standard deviation (n=6).

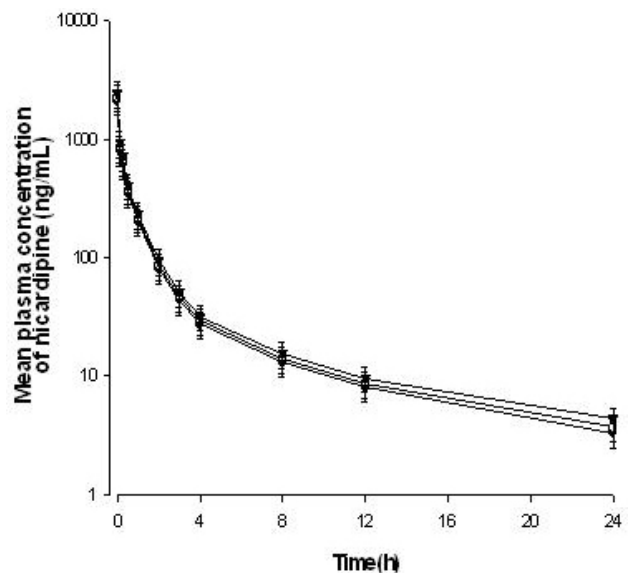


Fig. 4. Mean arterial plasma concentration-time profiles of nicardipine after its i.v. administration (4 mg/kg) without (●) or with 0.3 mg/kg (○) and 1.0 mg/kg (▼) of atorvastatin to rats. Bars represent the standard deviation (n=6).

P-gp. This result suggests that atorvastatin did not significantly inhibit P-gp activity.

Effect of atorvastatin on the pharmacokinetics of nicardipine.

The mean arterial plasma concentration-time profiles of oral nicardipine with or without atorvastatin are shown in Fig. 3 and 4. The relevant pharmacokinetic parameters of nicardipine are also listed in Table I and II.

The area under the plasma concentration-time curve (AUC) was significantly (1.0 mg/kg, $p < 0.05$) greater by 16.8-45.4%, and the peak concentration (C_{max}) was significantly (1.0 mg/kg, $p < 0.05$) higher by 28.0% with atorvastatin after oral administration of nicardipine. Consequently, the relative bioavailability (R.B.) of nicardipine was increased by 1.17- to 1.45-fold, and the absolute bioavailability (F) of nicardipine with atorvastatin was significantly (1.0 mg/kg, $p < 0.05$) increased by 16.7-20.9%, compared to that of the controls (14.3%). There was no significant change in the time to reach peak concentration (T_{max}) and the half-life ($t_{1/2}$) of nicardipine with atorvastatin.

Table I. Mean (\pm SD) pharmacokinetic parameters of nicardipine after its oral administration (12 mg/kg) with or without atorvastatin to rats

Parameter	Control without atorvastatin	Nicardipine + atorvastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng·h/ml)	416 \pm 86	486 \pm 108	605 \pm 148 ^a
C_{max} (ng/ml)	75 \pm 18	84 \pm 21	96 \pm 23 ^a
T_{max} (h)	0.46 \pm 10	0.46 \pm 0.10	0.58 \pm 0.20
$t_{1/2}$ (h)	8.5 \pm 1.7	9.1 \pm 1.9	9.9 \pm 2.4
A.B. (%)	14.3 \pm 3.0	16.7 \pm 3.1	20.9 \pm 4.2 ^a
R.B. (%)	100	117	145

Mean \pm SD (n=6), ^a $p < 0.05$, significant difference compared to controls, AUC: area under the plasma concentration-time curve from 0 h to time infinity, C_{max} : peak plasma concentration, T_{max} : time to reach peak concentration, $t_{1/2}$: terminal half-life, A.B. (%): absolute bioavailability, R.B. (%): relative bioavailability.

Table II. Mean (\pm SD) pharmacokinetic parameters of nicardipine after its intravenous administration (4 mg/kg) with or without atorvastatin to rats (Mean \pm SD) (n=6)

Parameter	Control without atorvastatin	Nicardipine + atorvastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng·h/ml)	969 \pm 204	1,044 \pm 226	1,138 \pm 259
CL (ml/min/kg)	51.6 \pm 12.5	47.9 \pm 10.4	43.9 \pm 9.3
$t_{1/2}$ (h)	6.9 \pm 1.4	7.1 \pm 1.6	7.4 \pm 1.8

AUC: area under the plasma concentration-time curve from time 0 to infinity, CL: total body clearance, $t_{1/2}$: terminal half-life.

The mean arterial plasma concentration-time profiles of i.v. nicardipine (4 mg/kg) with or without atorvastatin (0.3 or 1.0 mg/kg) are shown in Fig. 4. The relevant pharmacokinetic parameters of nicardipine are listed in Table II. Atorvastatin did not significantly change pharmacokinetic parameters of i.v. administration of nicardipine, suggesting that the metabolism of nicardipine by atorvastatin via hepatic CYP3A subfamily and renal excretion were almost negligible after intravenous administration in rats. Thus, enhanced oral bioavailability of nicardipine was due to increasing the intestinal absorption or reducing gut wall metabolism.

DISCUSSION

CYPs enzymes make a contribution significantly to the "first-pass" metabolism and oral bioavailability of many drugs. The "first-pass" metabolism of compounds in the intestine limits absorption of toxic xenobiotics and may ameliorate side effects. Moreover, induction or inhibition of intestinal CYPs may be responsible for significant drug and drug interactions when one agent decreases or increases the bioavailability and absorption rate constant of a concurrently administered drug (Kaminsky and Fasco, 1991).

Based on the broad overlap in the substrate specificities as well as co-localization in the small intestine, the primary site of absorption for orally administered drugs, CYP3A4 and P-gp have been recognized as a concerted barrier to the drug absorption (Cummins *et al.*, 2002; Benet *et al.*, 2003). Therefore, dual inhibitors against both CYP3A4 and P-gp should have a great impact on the bioavailability of many drugs where CYP3A4 metabolism as well as P-gp mediated efflux is the major barrier to the systemic availability and so could act synergistically to limit oral bioavailability of its substrates (Saeki *et al.*, 1993; Wachter *et al.*, 2001).

Studies on drug interactions with grapefruit juice have provided much understanding of the role of intestinal CYP450 in the absorption of orally administered drugs. CYP3A4 is the predominant P450 present in the small intestine (Kolars *et al.*, 1992). The inhibitory effect of atorvastatin on CYP3A4 activity is shown in Fig. 1. Atorvastatin inhibited CYP3A4 enzyme activity in a concentration-dependent manner and the 50% inhibition concentration (IC_{50}) values of atorvastatin on CYP3A4 activity was determined as 48 μ M. These results are consistent with the report (Lea and McTavish, 1997).

A cell-based P-gp activity test using rhodamine-123 also showed that atorvastatin did not significantly inhibit P-gp activity (Fig. 2). These results are not consistent with the

report (Holtzman *et al.*, 2006). The area under the plasma concentration-time curve (AUC) was significantly greater by 16.8-45.4%, and the peak concentration (C_{max}) was significantly higher by 28.0% with atorvastatin after oral administration of nicardipine. The absolute bioavailability (F) of nicardipine with atorvastatin was significantly increased by 16.7-20.9%, compared to that of the controls (14.3%).

Orally administered nicardipine is a substrate for CYP3A-mediated metabolism. The enhanced oral bioavailability of nicardipine by atorvastatin could be mainly due to inhibition of CYP3A subfamily in the intestine and/or in the liver rather than P-gp efflux in the intestine. Atorvastatin did not significantly change pharmacokinetic parameters of i.v. nicardipine, suggesting that atorvastatin did not inhibit the metabolism of nicardipine via hepatic CYP3A subfamily and renal excretion in rats. This result appeared to be consistent with a previous report that oral administration of atorvastatin significantly increased the oral bioavailability of verapamil in rats (Choi *et al.*, 2008).

The increased in bioavailability of orally administered nicardipine by atorvastatin might be due to inhibition of CYP3A subfamily rather than P-gp in the intestine, since the metabolism of nicardipine by atorvastatin via hepatic CYP3A subfamily and renal excretion were almost negligible after intravenous administration. These results suggest enhanced bioavailability of nicardipine may be mainly inhibited CYP3A metabolism in the intestine and /or in the liver by atorvastatin.

CONCLUSION

While there was no significant effect on the i.v. pharmacokinetics of nicardipine, atorvastatin significantly enhanced the oral bioavailability of nicardipine. Therefore, concomitant use of oral nicardipine and atorvastatin will require close monitoring for potential drug interactions.

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