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Effects of Atorvastatin and Lovastatin on the Bioavailability of Nicardipine in Rats

조선대학교 대학원

약학과

김 현 용

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아톨바스타틴 및 로바스타틴이 니카르디핀의 생체이용률에 미치는 영향

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이 논문을 약학박사학위신청 논문으로 제출함.

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Effects of Atorvastatin and Lovastatin on the Bioavailability of Nicardipine in Rats

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The aim of this study was to investigate the effect of atrovastatin or lovastatin on the bioavailabilty and pharmacokinetics of nicardipine after oral or intravenous administration of nicardipine in rats. Nicardipine was administered orally (12 mg/kg) or intravenously (i.v., 4 mg/kg) with atrovastatin or lovastatin (0.3 or 1.0 mg/kg) to rats, respectively. Compared to 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 45.4–53.7%, and the peak concentration (C_{max}) was significantly (1.0 mg/kg, $P < 0.05$) higher by 28.0–35.2% with atrovastatin or lovastatin after oral administration, respectively. Consequently, the relative bioavailability (R.B.) of nicardipine was increased by 1.17- to 1.54-fold, the absolute bioavailability (A.B.) of nicardipine with atrovastatin or lovastatin was 20.9–22.0%, which was significantly (1.0 mg/kg, $P < 0.05$) enhanced compared to

that of the controls (14.3%). Compared to the i.v. control, atrovastatin or lovastatin did not significantly change pharmacokinetic parameters of i.v. administration nicardipine.

The enhanced oral bioavailability of nicardipine suggests that CYP3A subfamily-mediated metabolism and P-gp-mediated efflux of nicardipine were inhibited in the intestine by atrovastatin or lovastatin.. Based on these results, modification of nicardipine of dosage regimen is required in the patients. Human studies are required to prove the above hypothesis.

Key words: Nicardipine; Atrovastatin; Lovastatin; CYP3A; P-gp; Bioavailability; Pharmacokinetics; Rats

국 문 초 록

아톨바스타틴 및 로바스타틴이 니카르디핀의 생체이용률에 미치는 영향

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고혈압 환자에서 아톨바스타틴 및 로바스타틴과 고혈압치료제인 니칼디핀과 병용처방이 가능하다. 아톨바스타틴 및 로바스타틴은 사이토크롬 P450 3A (CYP3A)와 P-당단백질 (P-gp)를 억제한다고 보고 되었다. 그러므로 아톨바스타틴과 로바스타틴이 CYP3A4 와 P-gp 의 기질인 니칼디핀의 생체이용률과 약물동태에 영향을 미칠 것으로 사료 되었다. 흰쥐에서 아톨바스타틴 및 로바스타틴 (0.3, 1.0 mg/kg)을 니칼디핀과 병용경구 (12 mg/kg)와 정맥 (4 mg/kg)으로 투여하여 본 연구를 실시하였다. 대조군에 비해 니카르디핀의 혈장농도곡선하면적 (AUC)과 최고혈중농도 (C_{max})는 아톨바스타틴 및 로바스타틴과 병용투여시 유의성 (1.0 mg/kg, $P < 0.05$) 있게 증가되였다. 대조군에

비해 니카르디핀의 절대적생체이용률(F)은 유의성 (1.0 mg/kg, $P < 0.05$) 있게 증가되었으며 상대적생체이용률은 1.19-1.79 배로 증가되었다.

정맥투여에서는 아톨바스타틴 및 로바스타틴은 약물동태학적 파라미터에 영향을 거의 주지못하였다.

이런결과는 아톨바스타틴 및 로바스타틴이 주로 소장에서 CYP3A4 와 P-당단백질을 억제시켜 니칼디핀의 생체이용률을 증가시킨 것으로 사료된다. 니카르디핀의 생체이용률에 미치는 영향으로는 로바스타틴이 아톨바스타틴 보다 많은 영향을 주었으며, 이런결과는 P-gp 에 대한 억제효과가 로바스타틴이 아톨바그타틴 보다 영향을 크게 주었기 때문으로 사료된다.

환자에서 니카르디핀과 아톨바스타틴 또는 니칼디핀과의 약물동태학적 연구가 필요하며 상기의 결과가 확증된다면 니칼디핀의 용량를 조절하는 것이 바람직 하다고 사료된다.

Part Ⅰ: Effects of Lovastatin on the Pharmacokinetics of Nicardipine after its Oral and Intravenous Administration to Rats

Abstract

The aim of this study was to investigate the effect of lovastatin on the pharmacokinetics of nicardipine after its oral and intravenous adminisration to rats. Nicardipine was administered orally (12 mg/kg) or intravenously (i.v., 4 mg/kg) without or with oral administration of lovastatin (0.3 or 1.0 mg/kg) to rats. Compared to the controls (given nicardipine alone) after oral administration of nicardipine with lovastatin, the area under the plasma concentration–time curve (AUC) of nicardipine was significantly (1.0 mg/kg, $P < 0.05$) greater by 53.7%, and the peak plasma concentration (C_{max}) was significantly (1.0 mg/kg, $P < 0.05$) higher by 35.2%. Consequently, compared to controls, the relative bioavailability (R.B.) of nicardipine was increased by 1.23- to 1.54-fold and the absolute bioavailability (A.B.) of nicardipine was significantly greater by 17.6–22.0%, respectively by lovastatin. Compared to i.v. controls, lovastatin did not significantly change pharmacokinetic parameters of i.v. administration nicardipine. The enhanced oral bioavailability of nicardipine by lovastatin suggests that CYP3A subfamily-mediated

metabolism and P-gp-mediated efflux of nicardipine were inhibited in the intestine. Based on these results, modification of nicardipine of dosage regimen is required in the patients. Human studies are required to prove the above hypothesis.

Key words: Nicardipine; Lovastatin; CYP3A subfamily; P-gp; Bioavailability; Pharmacokinetics; Rats

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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 [1,2]. 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 [3]. 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 [4.5]. It is a substrate of cytochrome P450 (CYP) 3A subfamily, especially CYP3A4 in humans and forms to pharmacologically inactive metabolite [6-8]. In addition, nicardipine is also a P-glycoprotein (P-gp) substrate [9,10].

Lovastatin, a 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitor, is widely used in preventing the progression of atherosclerosis by lowering plasma low-density lipid (LDL) levels in patients with hypercholesterolemia [11,12]. Lovastatin is mainly metabolized by CYP3A4 to a number of active metabolites [13,14]. Cytochrome P-450 oxidation is the primary route of phase I metabolism in humans and dogs [15]. Wang et al [16] reported that HMG-CoA reductase inhibitors (statins) are inhibitors of P-gp in the rodent system, but the effects of lovastatin on P-gp-inhibition are partially ambiguous. Thus, we attempted to

reevaluate P-gp activity using rhodamine-123 retention assay in P-gp-overexpressed MCF-7/ADR cells, and furthermore, to investigate the relationship between nicardipine, P-gp substrate and lovastatin.

Antihypertensive agents are commonly co-administered with cholesterol-lowering agents in the patients. 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 [17,18]. But there are fewer reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [19,20]. Moreover, lovastatin and nicardipine could be prescribed for the prevention or treatment of cardiovascular diseases as a combination therapy. Because lovastatin and nicardipine share the same pathways in their CYP3A-mediated metabolism, metabolism of nicardipine could be inhibited by lovastatin.

As a dual inhibitor of CYP3A4 and P-gp, lovastatin might affect the bioavailability and pharmacokinetics of nicardipine when lovastatin and nicardipine were used concomitantly for the prevention or therapy of cardiovascular diseases as a combination therapy. However, the effect of lovastatin on the pharmacokinetics of nicardipine has not been reported in vivo.

The low bioavailability of oral nicardipine is mainly due to pre-systemic metabolism and P-gp mediated efflux in the intestine. Lovastatin, a dual inhibitor of

CYP3A4 and P-gp, might improve the bioavailability of nicardipine in combination therapy, although adverse effects may occur if their doses are not adequate. Therefore, the aim of this study was to investigate the pharmacokinetics of nicardipine in the presence of lovastatin in rats.

Materials and Methods

Chemicals and apparatus

Nicardipine, lovastatin and nimodipine [internal standard for high-performance liquid chromatographic (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.

Apparatus used in this study were a HPLC equipped with a Waters 1515 isocratic HPLC pump, a Waters 717 plus autosampler, and a Waters™ 474 scanning fluorescence detector (Waters, Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic, Danbury, CT, USA), a vortex-mixer (Scientific Industries, NY, USA), and a high-speed micro centrifuge (Hitachi, Tokyo, Japan).

Animal experiments

Male Sprague–Dawley rats of 7–8 weeks old (270–300 g) were purchased from Dae Han Laboratory Animal Research Company (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Cocompany, 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 ± 2°C and a 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). 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 lightly ether. The left femoral artery and the left femoral vein were cannulated using polyethylene tubing (SP45; i.d. 0.58 mm, o.d. 0.96 mm; Natsume Seisakusho, 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 ^{o}C for 30 min; 3.0 mL/kg) without (control) or with 0.3 or 1.0 mg/kg of oral lovastatin, and an i.v. group (4 mg/kg of nicardipine, dissolved in 0.9% NaCl injectable solution; homogenized at 36 ^{o}C for 30 min; 1.5 mL /kg) without (control) or with 0.3 or 1.0 mg/kg of oral lovastatin. Nicardipine was administered orally using a gastric gavage tube, and lovastatin was orally administered 30 min prior to oral or intravenous administration of nicardipine. Nicardipine was injected over 0.5 min through the femoral vein. A blood sample (approximately 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. A blood sample was centrifuged (13,000 rpm, 5 min), and a plasma sample was stored at –40˚C until the HPLC analysis of nicardipine. Approximately 1 mL of whole blood collected from untreated 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.* [21]. Briefly, a 50-μL of nimodipine (2 μg/mL), a 20-μL of 2 N sodium hydroxide solution, and 1.2-mL of tert-butylmethylether : hexane (75:25) were added to 0.2-mL of a 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^{o}C. The residue was dissolved in 200-μL of the mobile phase and centrifuged (13,000 rpm, 5 min). A 50-μL of the supernatant was injected directly onto the HPLC system. Chromatographic separations were achieved using a Symmetry® C_{18} column (4.6 × 150 mm, 5 μm; Waters), and a μBondapak™ C_{18} HPLC Precolumn (10 μm, Waters). The mobile phase was acetonitrile : 0.015 M KH_2PO_4 (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 (Figure 1). The detection limit of nicardipine in rat's plasma was 5 ng/mL. The coefficients of variation were below 14.1% (Figure 2).

Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated using 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}) 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 (A.B.%) 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 $AUC_{with\ lovastatin}/AUC_{control} \times 100$.

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 ℃ for 30 min. After the incubation of the cells with 20-μM rhodamine-123 in the presence or absence of lovastatin (1, 3 or 10 μM) or verapamil (100 μM) 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 controls.

Statistical analysis

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

Results

The mean arterial plasma concentration–time profiles of oral nicardipine

(12mg/kg) in the presence (0.3 or 1.0 mg/kg) or absence of lovastatin are shown in Figure 3. The relevant pharmacokinetic parameters of nicardipine are also listed in Table 3.

The area under the plasma concentration–time curve (AUC) was significantly (1.0 mg/kg, $P < 0.05$) greater by 53.7%, and peak plasma concentration (C_{max}) was significantly (1.0 mg/kg, $P < 0.05$) higher by 35.2% than those of without lovastatin after oral administration of nicardipine. Consequently, the relative bioavailability (R.B.) of nicardipine was significantly (1.0 mg/kg, $P < 0.05$) increased by 1.23- to 1.54-fold, and the absolute bioavailability (F) of nicardipine with lovastatin was significantly (1.0 mg/kg, $P < 0.05$) increased by 17.6–22.0%, compared to that of the controls (14.3%). In this study, the cell-based P-gp activity test using rhodamine-123 also showed that lovastatin (10 μM, $P < 0.01$) significantly inhibited P-gp activity (Fig. 5).

This suggests that lovastatin inhibited the P-gp in rats. There was no significant change in the time to reach peak plasma concentration (T_{max}) and the half-life ($t_{1/2}$) of nicardipine with lovastatin.

The mean arterial plasma concentration–time profiles of i.v. nicardipine (4 mg/kg) in the presence (o.3 or 1.0) or absence of lovastatin are shown in Figure 4. The relavant pharmacokinetic parameters of nicardipine are also listed in Table 4. Lovastatin did not significantly change the pharmacokinetic parameters of i.v. administration of nicardipine, suggesting that lovastatin did not inhibit the hepatic

metabolism of nicardipine via CYP3A subfamily in rats. Consequently, lovastatin may improve the oral bioavailability of nicardipine by increasing the small 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 rat constant of a concurrently administered drug [22].

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 [23,24]. 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 (F)of its substates [24,25].

. Besides the extensive metabolism by CYP3A4, nicardipine appeared to be the substrate of P-gp, thus, lovastatin is a inhibitor of P-gp and CYP3A4, it could act synergistically to increase the oral bioavailability (F) of nicardipine.

. Besides the extensive metabolism by CYP3A4, nicardipine appeared to be the substrate of P-gp, suggesting that P-gp and CYP3A4 could act synergistically to limit oral bioavailability (F) of nicardipine [25,26].

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 [27]. A cell-based P-gp activity test using rhodamine-123 also showed that lovastatin (10 μM, $P < 0.01$) significantly inhibited P-gp activity (Fig. 5). These results are consistent with the report [16] that lovastatin is an inhibitor of P-gp.

Orally administered nicardipine is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux. The enhanced oral bioavailability of nicardipine by lovastatine could be due to the inhibition of P-gp. Lovastatin did not significantly change pharmacokinetic parameters of i.v. nicardipine, suggesting that lovastatin did not inhibit the metabolism of nicardipine via hepatic CYP3A subfamily in rats. This result appeared to be consistent with a previous report that oral administration of atrovastatin and fluvastatin significantly increased the oral bioavailability (F) of verapamil or diltiazem in rats [19,20]. Resveratrol and morin significantly increased AUC and C_{max} nicardipine in rats [28,29]

The increase in bioavailability (F) of orally administered nicardipine by lovastatin might be due to the inhibition of CYPs and P-gp in the intestine. Metabolism of nicardipine by lovastatin via hepatic CYP3A subfamily was almost negligible after intravenous administration of nicardipine. These results suggest enhanced bioavailability of nicardipine must be mainly inhibited P-gp efflux and CYP3A metabolism in the intestine by lovastatin.

Conclusion

While there was no significant effect on the i.v. pharmacokinetics of nicardipine,

lovastatin (1.0 mg/kg) significantly enhanced the oral bioavailability (F) of nicardipine. Therefore, concomitant use of oral lovastatin and nicardipine will require close monitoring for potential drug interactions.

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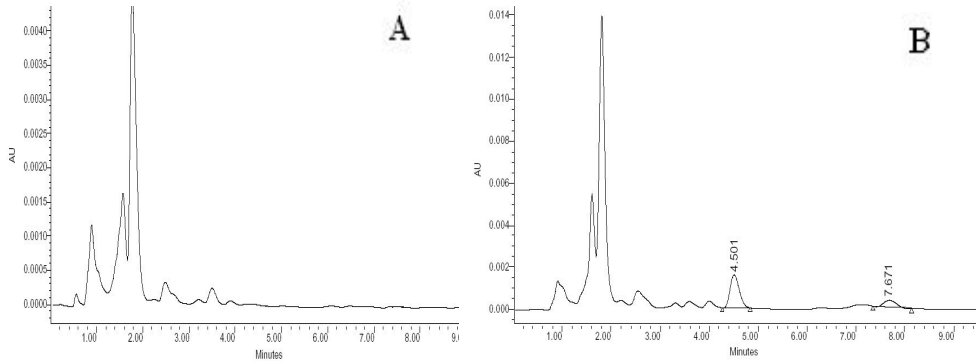

Figure 1. HPLC chromatograms of the rat's blank plasma (A) and plasma spiked (B) with internal standard (IS, 4.5 min) and nicardipine (7.7 min).

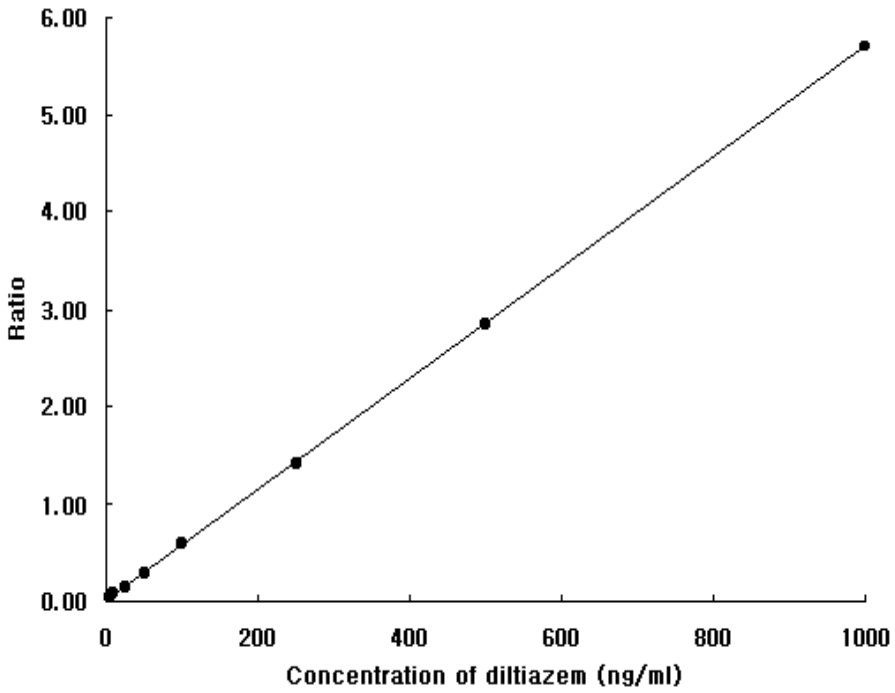

Figure 2. An example of a calibration curve of nicardipine in rat's plasma.

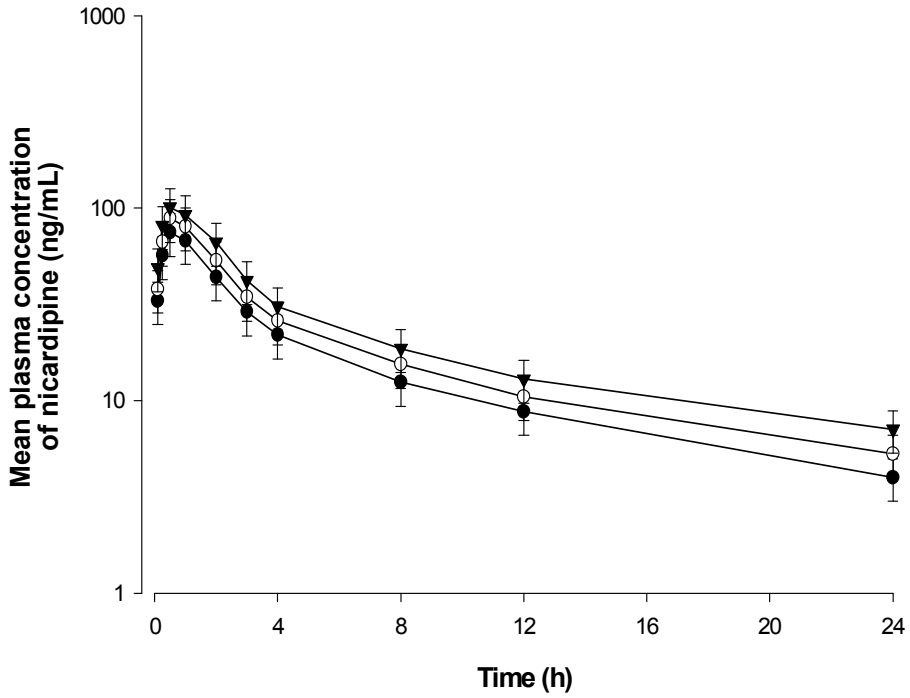

Figure 3. Mean arterial plasma concentration–time profiles of nicardipine after its oral administration (12 mg/kg) without (●) or with 0.3 mg/kg (○) and 1.0 mg/kg (▼) of lovastatin to rats. Bars represent the standard deviation (n = 6).

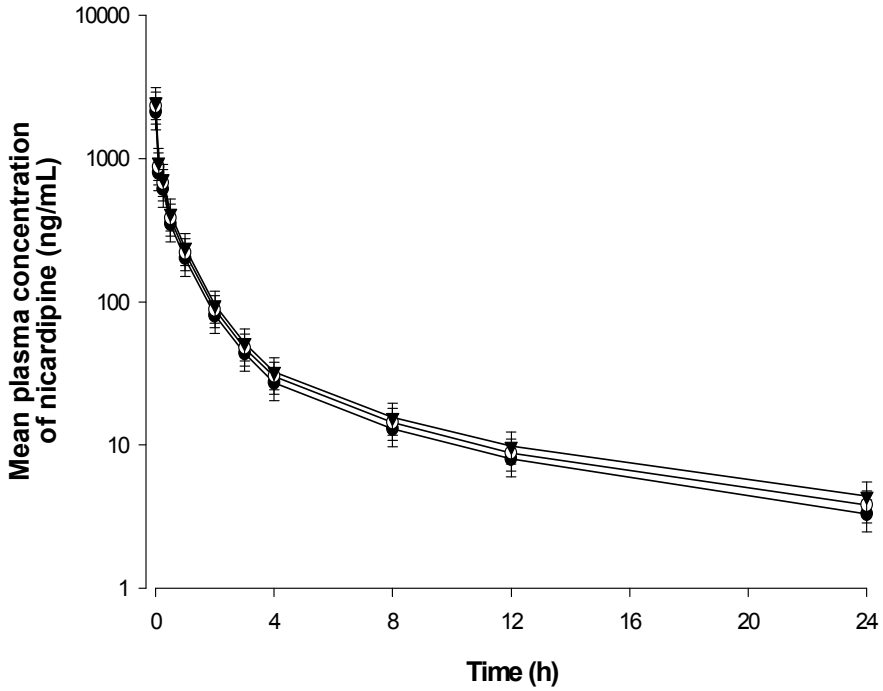

Figure 4. Mean arterial plasma concentration–time profiles of nicardipine after its i.v. administration (4 mg/kg) without (●) or with 0.3 mg/kg (○) and 1.0 mg/kg (▼) of lovastatin to rats. Bars represent the standard deviation (n = 6).

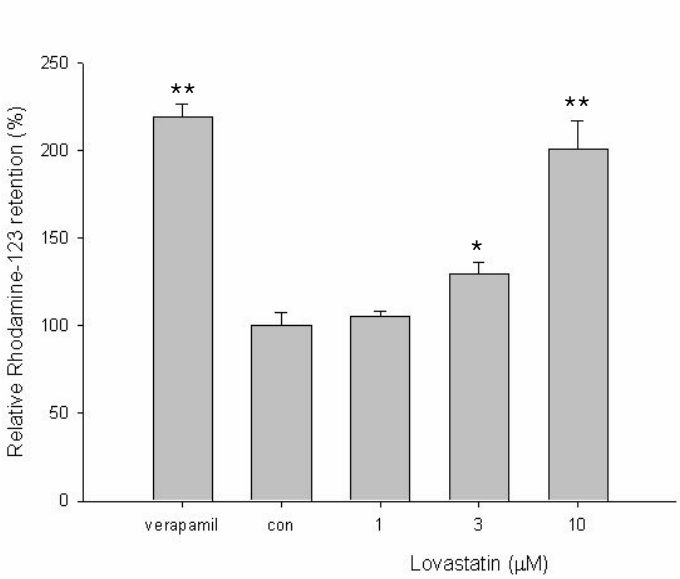

Figure 5. Rhodamine-123 (R-123) retention in lovastatin-treated MCF-7/ADR cells. After the incubation of MCF-7/ADR cells with 20 M R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by total protein contents of each sample. Data represents means ± SEM of 4 separate samples (significant versus the control MCF-7 cells, **P < 0.01). Verapamil (100 μM) was used as a positive control.

Table 1. Mean arterial plasma concentrations of nicardipine after its oral administrat ion (12 mg/kg) with or without lovastatin to rats (mean ± SD, n = 6).

Time (h)	Control (without lovastatin)	Nicardipine with lovastatin 0.3 mg/kg	Nicardipine with lovastatin 1.0 mg/kg
0	0	0	0
0.1	33.1 ± 6.4	38.0 ± 7.1	49.1 ± 10.3
0.25	57.0 ± 11.5	66.9 ± 13.9	81.3 ± 17.2
0.5	75.0 ± 15.1	88.5 ± 16.5	101.4 ± 23.3
1	68.0 ± 13.7	80.3 ± 16.3	92.6 ± 19.0
2	44.0 ± 9.2	53.4 ± 10.4	66.9 ± 13.7
3	29.0 ± 5.9	34.5 ± 6.8	42.2 ± 7.9
4	22.0 ± 4.4	26.0 ± 5.4	30.9 ± 5.6
8	12.5 ± 2.3	15.5 ± 2.8	18.7 ± 3.7
12	8.8 ± 1.8	10.5 ± 2.1	13.0 ± 2.8
24	4.0 ± 0.8	5.3 ± 1.1	7.1 ± 1.4

Table 2. Mean arterial plasma concentrations of nicardipine after its intravenous ad ministration (4 mg/kg) with or without lovastatin to rats (mean ±SD, n = 6).

Time (h)	Control (without lovastatin)	Nicardipine with lovastatin 0.3 mg/kg	Nicardipine with lovastatin 1.0 mg/kg
0	2018.0 ± 363.1	2316.0 ± 441.6	2489.0 ± 506.0
0.1	793.0 ± 162.5	871.1 ± 184.2	939.0 ± 197 .0
0.25	611.0 ± 112.5	671.0 ± 126.8	725.0 ± 135.0
0.5	349.0 ± 64.4	383.0 ± 73.7	415.0 ± 84.1
1	201.0 ± 37.1	220.0 ± 42.2	239.0 ± 48.3
2	80.0 ± 15.8	88.0 ± 17.2	94.7 ± 19.6
3	43.6 ± 8.7	47.5 ± 10.1	51.7 ± 12.5
4	27.2 ± 6.5	30.2 ± 7.5	32.5 ± 8.0
8	13.0 ± 3.4	14.4 ± 3.7	15.6 ± 4.0
12	8.0 ± 2.3	8.8 ± 2.3	9.8 ± 2.8
24	3.3 ± 0.8	3.8 ± 1.0	4.4 ± 1.2

Table 3. Mean (± S.D.) pharmacokinetic parameters of nicardipine after its oral administration (12 mg/kg) with or without lovastatin to rats.

Paramater	Control (without lovastatin)	Nicardipine+ lovastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng·h/mL)	416.0 ± 86.1	512.1 ± 102.0	639.3 ± 122.1*
C_{max} (ng/mL)	75.0 ± 18.1	88.5 ± 21.0	101.4 ± 23.1*
T_{max} (h)	0.46 ±0.10	0.58 ± 0.20	0.58 ± 0.20
$t_{1/2}$ (h)	8.5 ± 1.7	9.1 ± 2.0	9.7 ± 2.6
A.B. (%)	14.3 ± 3.1	17.6 ± 4.0	22.0 ± 4.3*
R.B. (%)	100	123	154

Mean ± S.D. (n=6), * $P < 0.05$, 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 (F)

R.B. (%): relative bioavailability.

Table 4. Mean (± S.D.) pharmacokinetic parameters of nicardipine after its intravenous administration (4 mg/kg) with or without lovastatin to rats.

Paramater	Control (without lovastatin)	Nicardipine+ lovastatin	
		0.3 mg/kg	1.0 mg/kg
AUC(ng·h/mL)	969 ± 204	1068 ± 218	1168 ± 246
CL(mL/min/kg)	51.6 ± 12.5	46.8 ± 10.6	43.0 ± 9.2
$t_{1/2}$(h)	6.9 ± 1.4	7.1 ± 1.5	7.4 ± 1.7

Mean ± S.D. (n=6)

AUC: area under the plasma concentration-time curve from time 0 to time infinity

CL: total body clearance;

$t_{1/2}$: terminal half-life

Part Ⅱ: Effects of Atorvastatin on the Pharmacokinetics of Nicardipine after its Oral and Intravenous Administration to Rats

Abstract

The aim of this study was to investigate the effect of atrovasatatin 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 atrovasatatin (0.3 or 1.0 mg/kg) to rats. 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 atrovasatatin was significantly greater

by16.7–20.9% compared to that of the controls (14.3%). Compared to the i.v. control, atrovasatatin 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 and P-gp-mediated efflux of nicardipine were inhibited in the intestine. Based on these results, modification of nicardipine of dosage regimen is required in the patients. Human studies are required to prove the above hypothesis.

Key words: Nicardipine; Atrovasatatin; 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 [1,2] 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 [3]. 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 [4.5]. It is a substrate of cytochrome P450 (CYP) 3A subfamily, especially CYP3A4 in humans and forms to pharmacologically inactive metabolite [6-8]. In addition, nicardipine is also a P-glycoprotein (P-gp) substrate [9,10].

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 [11]. Atorvastatin has been identified as a substrate of CYP3A4 which could be

controlling factors for the low systemic availability of atorvastatin [12]. Accordingly, previous studies have revealed that many CYP3A4 inhibitors including itraconazole, clarithromycin, azithromycin, nelfinavir and grapefruit juice increased the bioavailability of atorvastatin [13]. Holtzman et al [14] reported that HMG-CoA reductase inhibitors (statins) are inhibitors of P-gp in the rodent system, but the effects of atorvastatin on P-gp-inhibition are partially ambiguous. Thus we attempted to reevaluate P-gp activity using rhodamine-123 retention assay in P-gp-overexpressed MCF-7/ADR cells, and furthermore, to investigate the relationship between nicardipine, P-gp substrates and atrovastatin.

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 [15] 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 [13]. 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 [16,17]. But there are fewer reports about the effects of HMG-CoA reductase

inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [18,19]. 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 and P-gp mediated efflux in the intestine. Atorvastatin, a dual inhibitor of CYP3A4 and P-gp, might improve the bioavailability of nicardipine in combination therapy. 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, atrovasatatin 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 Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (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 ± 2°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 atrovasatatin, 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 atrovasatatin. Nicardipine was administered orally using a gastric

gavage tube, and atrovasatatin 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 untreated 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) [20]. Briefly, a 50-μL aliquot of nimodipine (2 μg/mL), a 20-μL 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^{o}C. The residue was dissolved in 200-μL of the mobile phase and centrifuged (13,000 rpm, 5 min). A 50-μL aliquot of the supernatant was injected into the HPLC system. Chromatographic

separations were achieved using a Symmetry® C_{18} column (4.6 × 150 mm, 5 μm, Waters), and a μBondapak™ C_{18} HPLC Precolumn (10 μm, Waters). The mobile phase was acetonitrile:0.015 M KH_2PO_4 (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 (Figure 6). The detection limit of nicardipine in rat's plasma was 5 ng/mL. The coefficients of variation for nicardipine were below 14.1% (Figure 7).

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 $AUC_{\text{with atrovasatatin}}/AUC_{control} \times 100$.

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-μM rhodamine-123 in the presence or absence of atorvastatin (1, 3 or 10 μM) or verapamil (100 μM) 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.

Statistical analysis

All data are expressed with their standard deviation (mean ± S.D.). 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

The mean arterial plasma concentration–time profiles of oral nicardipine with or without atrovasatatin are shown in Figure 8 and 9. The relevant pharmacokinetic parameters of nicardipine are also listed in Table 7 and 8.

Figure 8 showed the plasma concentration–time profiles of nicardipine after oral administration at a dose of 12 mg/kg of nicardipine in rats with or without atrovasatatin (0.3 or 1.0 mg/kg), and the pharmacokinetic parameters of oral nicardipine are summarized in Table 7. 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 atrovasatatin 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 atrovasatatin was significantly (1.0 mg/kg, $P < 0.05$) increased by 16.7.–20.9%, compared to that of the controls (14.3%). In this study, the cell-based P-gp activity test using rhodamine-123 also showed that atorvastatin (10 μM, $P < 0.01$) significantly inhibited P-gp activity (Fig. 10). This sugges that atorvastatin inhibited the P-gp. There was no significant change in the time to reach peak concentration (T_{max}) and the half-life ($t_{1/2}$) of

nicardipine with atrovasatatin.

The mean arterial plasma concentration–time profiles of i.v. nicardipine(4 mg/kg) with or without atrovasatatin(0.3 or 1.0 mg/kg) are shown in Figure 9. The relevant pharmacokinetic parameters of nicardipine are listed in Table 8. Figure 9 showed the plasma concentration–time profiles of nicardipine after i.v. (4 mg/kg) without or with of atrovasatatin (0.3 or 1.0 mg/kg) to rats. As shown in Table 8, atrovasatatin did not significantly change pharmacokinetic parameters of i.v. administration of nicardipine, suggesting that atrovasatatin did not inhibit the hepatic metabolism of nicardipin via CYP3A subfamily 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 rat constant of a concurrently administered drug [21].

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 [22,23]. 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 (F)of its substates [24,25].

. Besides the extensive metabolism by CYP3A4, nicardipine appeared to be the substrate of P-gp, thus, atorvastatin is a inhibitor of P-gp and CYP3A4, it could act synergistically to increase the oral bioavailability (F)of nicardipine.

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 [26]. A cell-based P-gp activity test using rhodamine-123 also showed that atorvastatin (10 μM, $P < 0.01$) significantly inhibited P-gp activity (Fig. 10). These results are consistent with the report [14] that atorvastatin is an inhibitor of P-gp.

Orally administered nicardipine is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux. The enhanced oral bioavailability of nicardipine by lovastatin could be mainly due to inhition of P-gp efflux in the intestine. Atrovasatatin did not significantly change pharmacokinetic parameters of i.v. nicardipine, suggesting that lovastatin did not inhibit the metabolism of nicardipine via hepatic CYP3A subfamily in rats.This result appeared to be consistent with a previous report that oral administration of atrovasatatin significantly increased the oral bioavailability (F) of verapamil in rats [19].

The increased in bioavailability of orally administered nicardipine by atorvastatin might be due to inhibition of P-gp in the intestine, since the metabolism of nicardipine by atorvastatin via hepatic CYP3A subfamily was almost negligible after intravenous administration. These results suggest enhanced bioavailability of nicardipine must be mainly inhibited P-gp efflux and CYP3A metabolism in the intestine by atrovasatatin.

Conclusion

While there was no significant effect on the i.v. pharmacokinetics of nicardipine, atrovasatatin (0.3 or 1.0 mg/kg) significantly enhanced the oral bioavailability (F) of nicardipine. Therefore, concomitant use of oral nicardipine and atrovasatatin will require close monitoring for potential drug interactions.

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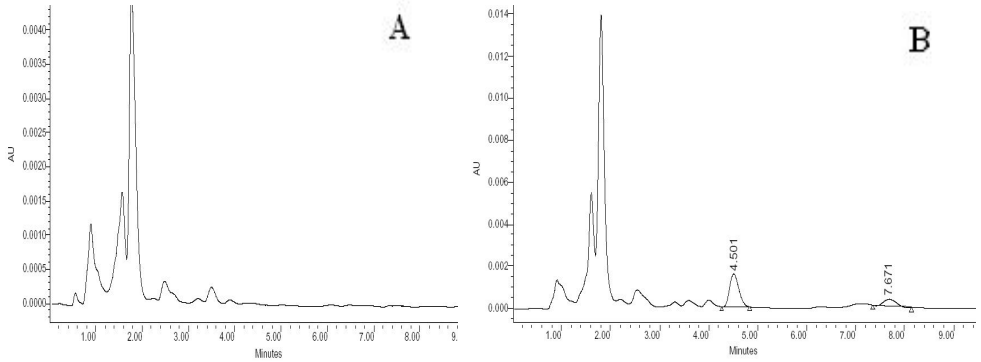

Figure 6. HPLC chromatograms of the rat's blank plasma (A) and plasma spiked (B) with internal standard (IS, 4.5 min) and nicardipine (7.7 min).

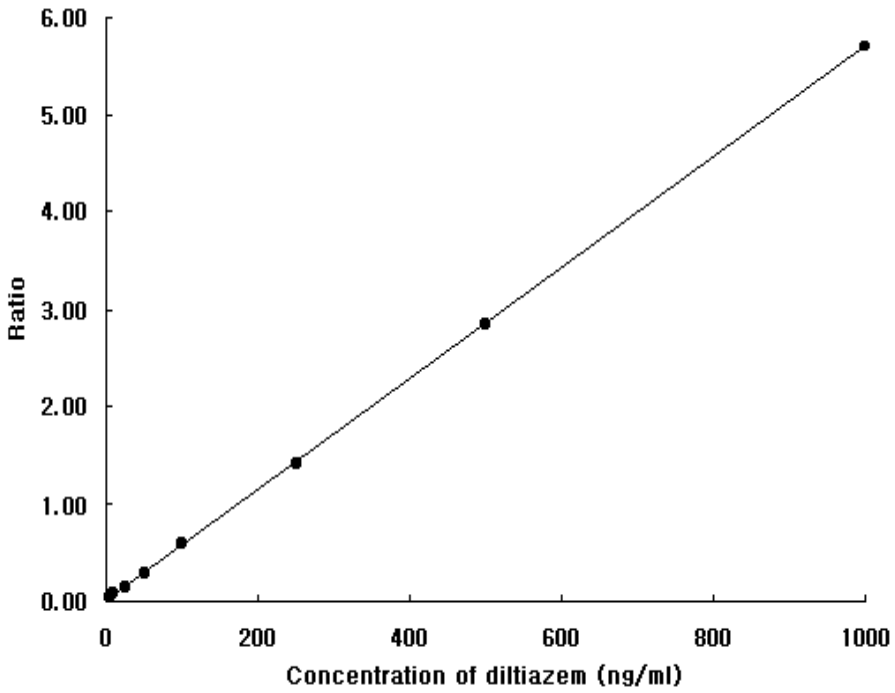

Figure 7. A calibration curve of nicardipine when spiked into the rat's blank plasma.

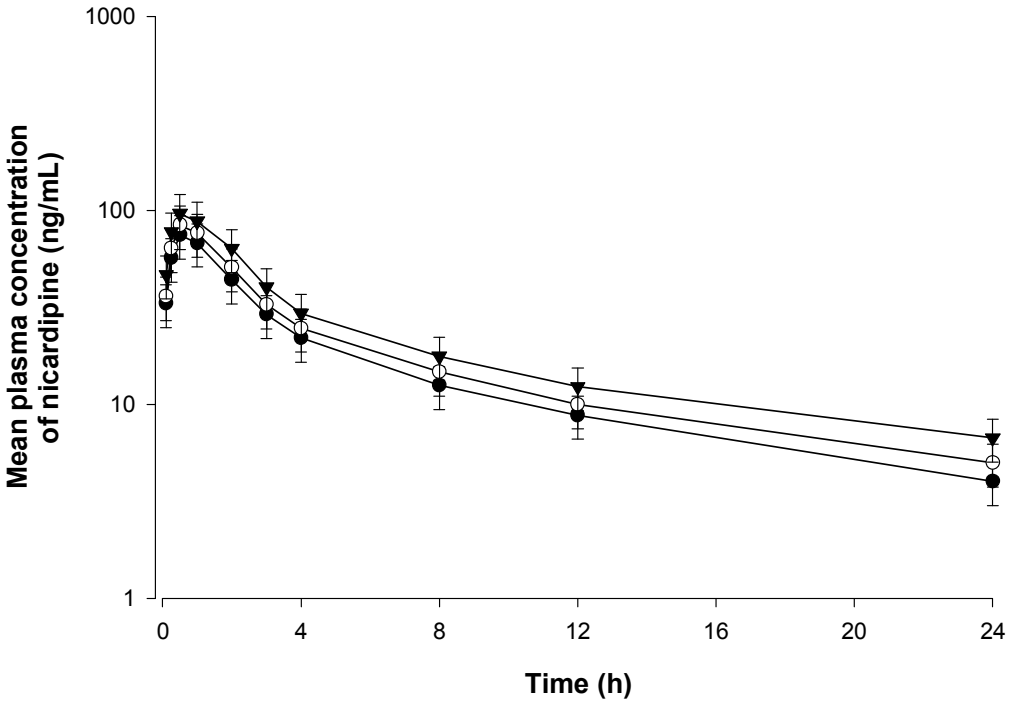

Figure 8. Mean arterial plasma concentration–time profiles of nicardipine after its oral administration (12 mg/kg) without (●) or with 0.3 mg/kg (○) and 1.0 mg/kg (▼) of atrovasatatin to rats. Bars represent the standard deviation (n = 6).

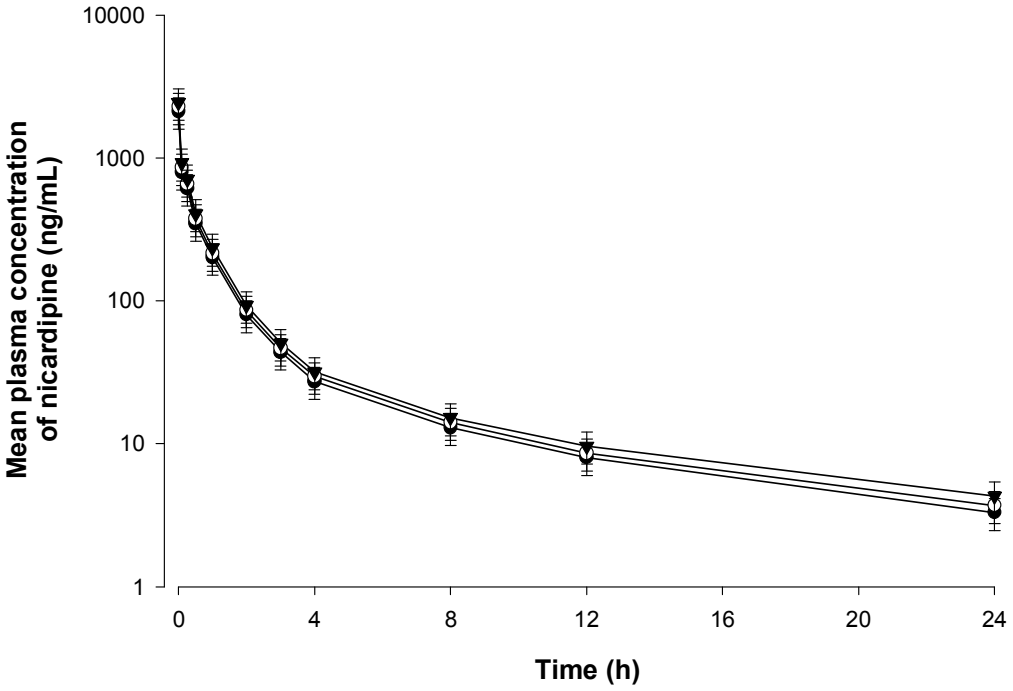

Figure 9. Mean arterial plasma concentration–time profiles of nicardipine after its i.v. administration (4 mg/kg) without (●) or with 0.3 mg/kg (○) and 1.0 mg/kg (▼) of atrovasatatin to rats. Bars represent the standard deviation (n = 6).

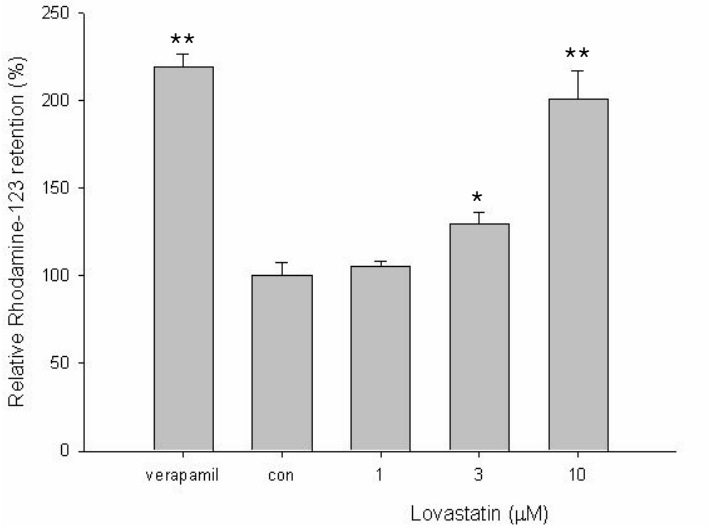

Figure 10. Rhodamine-123 (R-123) retention in atorvastatin-treated MCF-7/ADR cells. After incubation of MCF-7/ADR cells with 20 M R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by total protein contents of each sample. Data represents means ± SEM of 4 separate samples (significant versus the control MCF-7 cells, **P < 0.01). Verapamil (100 μM) was used as a positive control.

Table 5. Mean arterial plasma concentrations of nicardipine after its oral administration (12 mg/kg) with or without atrovasatatin to rats (mean ± SD, n = 6).

Time (h)	Control without atorvastatin	Nicardipine with atorvastatin 0.3 mg/kg	Nicardipine with atorvastatin 1.0 mg/kg
0	0	0	0
0.1	33.1 ± 6.4	36.1 ± 6.9	46.6 ± 10.1
0.25	57.0 ± 11.5	63.5 ± 13.6	77.2 ± 16.6
0.5	75.0 ± 15.1	84.1 ± 16.3	96.3 ± 22.8
1	68.0 ± 13.7	76.3 ± 16.0	88.0 ± 18.5
2	44.0 ± 9.2	50.7 ± 10.2	63.5 ± 13.4
3	29.0 ± 5.9	32.7 ± 6.7	40.1 ± 7.6
4	22.0 ± 4.4	24.7 ± 5.3	29.4 ± 5.6
8	12.5 ± 2.3	14.7 ± 2.6	17.7 ± 3.5
12	8.8 ± 1.8	10.0 ± 2.0	12.3 ± 2.6
24	4.0 ± 0.8	5.0 ± 1.0	6.7 ± 1.3

Table 6. Mean arterial plasma concentrations of nicardipine following its intravenous administration (4 mg/kg) with or without atorvastatin to rats (mean ±SD, n = 6).

Time (h)	Control without atorvastatin	Nicardipine with atorvastatin 0.3 mg/kg	1.0 mg/kg
0	2018.0 ± 363.1	2270.0 ± 434.6	2439.0 ± 496.2
0.1	793.0 ± 162.5	853.0 ± 182.9	920.0 ± 187.1
0.25	611.0 ± 112.5	657.0 ± 120.1	710.0 ± 130.6
0.5	349.0 ± 64.4	375.0 ± 69.9	406.0 ± 81.1
1	201.0 ± 37.1	215.0 ± 38.9	234.0 ± 44.3
2	80.0 ± 15.8	86.1 ± 16.6	92.8 ± 19.1
3	43.6 ± 8.7	46.4 ± 9.2	50.6 ± 11.9
4	27.2 ± 6.5	29.5 ± 7.0	31.8 ± 7.8
8	13.0 ± 3.4	14.1 ± 3.5	15.2 ± 3.9
12	8.0 ± 2.3	8.6 ± 2.4	9.6 ± 2.6
24	3.3 ± 0.8	3.7 ± 1.0	4.3 ± 1.2

Table 7. Mean (± S.D.) pharmacokinetic parameters of nicardipine after its oral administration (12 mg/kg) with or without atrovasatatin to rats.

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

Mean ± S.D. (n=6), * $P < 0.05$, 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 8. Mean (± S.D.) pharmacokinetic parameters of nicardipine after its intravenous administration (4 mg/kg) with or without atrovasatatin to rats.

Paramater	Control without atrovasatatin	Nicardipine+ atrovasatatin	
		0.3 mg/kg	1.0 mg/kg
AUC(ng·h/mL)	969 ± 204	1044 ± 226	1138 ± 259
CL(mL/min/kg)	51.6 ± 12.5	47.9 ± 10.4	43.9 ± 9.3
$t_{1/2}$(h)	6.9 ± 1.4	7.1 ± 1.6	7.4 ± 1.8

Mean ± S.D. (n=6)

AUC: area under the plasma concentration-time curve from time 0 to infinity

CL: total body clearance;

$t_{1/2}$: terminal half-life

감사의 글

부족한 저를 항상 이끌어 주시고 격려해주신 최준식 지도교수님께 진심으로 감사드립니다. 그리고 논문 자구 수정과 심사를 해주신 한효경 교수님께 감사를 드립니다. 지금까지 부족한 저를 인내와 사랑으로 항상 기도해준 사랑하는 아내에게 마음속 깊이 감사를 드립니다. 그리고 논문실험에 도움을 주신 이성 선생님과 이정록 선생님을 비롯한 약제학교실원 여러분께도 감사를 드립니다. 앞으로 사회에 나가서 지금의 가르침을 바탕삼아 모든 일에 최선을 다하고 하나님의 영광을 위하여 사랑과 봉사하는 약사가 되겠습니다.

박사학위가 나오기 까지 저를 인도하신 하나님께 영광을 돌립니다.

저작물 이용 허락서

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논문제목	한글: 아톨바스타틴 및 로바스타틴이 니카르디핀의 생체이용률에 미친 영향 영문: Effects of atorvastatin and lovastatin on the bioavailability of nicardipine in rats				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

– 다 음 –

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의 (√) 반대 ( )

2009 년 06 월

저작자: 김현용 (서명 또는 인)

조선대학교 총장 귀하