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박사학위논문

Effects of HMG-CoA reductase inhibitors on the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats

조선대학교 대학원

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흰쥐에서 HMG-CoA 환원효소억제제가 니페디핀 및 그대사체인 디히드로니페디핀의 약물동태에 미치는 영향

2014 년 1 월 10 일

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이 논문을 의학박사학위신청 논문으로 제출함.

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조선대학교 대학원

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항콜레스테롤제와 항고혈압치료제인 니페디핀과의 병용투여가 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 많다. 그러므로 이에 대한 상호작용을 연구하고자 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 HMG-CoA 환원효소 억제제인 아톨바스타틴 (0.3, 1.0 mg/kg), 프루바스타틴 (0.3, 1.0 mg/kg), 프라바스타틴 (0.3, 1.0 mg/kg)과 심바스타틴 (0.3, 1.0 mg/kg)을 병용 경구 및 정맥 투여한 후 니페디핀 및 그 주요 대사체인 디히드로니페디핀의 약물동태학적 변수들을 대조군과 비교 검토하였다. 그리고 HMG-CoA 환원효소 억제제인 아톨바스타틴, 프루바스타틴, 프라바스타틴과 심바스타틴이 cytochromeP450(CYP)3A4 활성과 P-glycoprotein(P-gp)의 활성에 미치는 영향도 평가 하였다. 프루바스타틴과 심바스타틴이 CYP3A4 활성과 P-gp 의 활성을 유의성 있게 억제시켰다.

프루바스타틴 또는 심바스타틴과 각각 병용 투여시 니페디핀의 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 프루바스타틴 (1.0 mg/kg) 또는 심바스타틴 (0.3, 1.0 mg/kg)과의 병용투여군에서 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성 ($P < 0.05$, $P < 0.01$) 있게 증가시켰으나, 아톨바스타틴과 프라바스타틴과 병용투여군에서는 증가시켰으나 유의성은 없었다. HMG-CoA 환원효소 억제제의

저농도(0.3 mg/kg) 에서는 증가는 시켰으나 유의성은 없었다.

프루바스타틴 또는 심바스타틴(0.3, 1.0 mg/kg)과의 병용투여군에서 니페디핀의 전신클리어런스 (CL/F)는 유의성 ($P < 0.05$, $P < 0.01$) 있게 각각 감소되었다.

절대적생체이용률 (AB)도 대조군에 비해 각각 유의성 ($P < 0.05$) 있게 증가되었다. 프루바스타틴 (1.0 mg/kg) 또는 심바스타틴 (1.0 mg/kg)과 니페디핀을 병용투여한군에서 대조군에 비해 대사체인 디히드로니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 ($P < 0.05$) 있게 증가되었다. 그리고 프루바스타틴 또는 심바스타틴과 병용투여군에서 니페디핀의 대사율 (MR)을 유의성 ($P < 0.05$) 있게 감소시켰다.

정맥투여군에서는 아톨바스타틴, 프루바스타틴, 프라바스타틴과 심바스타틴은 니페디핀의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 항콜레스테롤제인 프루바스타틴 또는 심바스타틴을 각각 고혈압치료제인 니페디핀과 병용투여 하였을 때 경구투여된 니페디핀의 생체이용률이 유의성 있게 증가된 것은 프루바스타틴 및 심바스타틴에 의해서 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 소장과 간장에 존재하는 CYP3A 억제에 의한 니페디핀의 초회통과효과 (대사)감소와 전신클리어런스 감소에 기인한 것으로 사료된다.

항콜레스테롤제인 HMG-CoA 환원효소 억제제와 (특히 프루바스타틴과 심바스타틴) 항고혈압치료제인 니페디핀과의 병용투여시 이들의 상호작용을 고려하는 것이 중요하다고 사료 되어진다.

A. **Introduction**

Statins such as atorvastatin, fluvastatin, pravastatin and simvastatin inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which leads to a decrease in circulating total cholesterol and low density lipoprotein cholesterol (LDL-C) concentrations [1]. They are widely used to treat hypercholesterolemia by lowering plasma low density lipoprotein (LDL) levels and also have antioxidant, anti-inflammatory and antithrombotic properties [1]. Hyperchoesterolemia is one of the risk factors of patients suffering from cardiovascular disease such as hypertension and ischemic heart disease. Recently, some preliminary observations point to beneficial effects of statins on blood pressure and the prognosis for hypertensive and dyslipidemic patients [1, 2].

The oxidative biotransformations of simvastatin [3], fluvastatin [4] and pravastatin [5] are mediated primarily by cytochrome P450 (CYP) 3A4. Atorvastatin has been identified as a substrate of CYP3A4, which could be a controlling factor for the low systemic availability of atorvastatin [6]. Moreover, it was reported that statins are inhibitors of P-gp in rodent system [7]. However, the effects of atorvastatin, fluvastatin, pravastatin and simvastatin on the inhibition of CYP3A4 and P-gp activity are somewhat ambiguous. Thus, we attempted to reevaluate P-gp activity using the rhodamine-123 retention assay in P-gp-overexpressing MCF-7/ADR cells and assessed CYP3A4 activity.

Nifedipine (dimethyl2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a calcium channel-blocking agent that is widely used for the treatment of essential hypertension, coronary artery spasm, and angina pectoris [8]. It inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically plugging the channel, resulting in decreased intracellular calcium levels, inhibition of the contractile processes of smooth muscle cells, dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, and decreased total peripheral resistance, systemic blood pressure, and afterload [9, 10].

In humans, nifedipine is predominantly metabolized by CYP3A4 to its primary pyridine metabolite, dehydronifedipine [11, 12]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs [13]. CYP3A4 is the most abundant CYP enzyme (30-40%) in adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam, and erythromycin [14, 15]. There are some reports that nifedipine is a substrate of CYP3A4 in human [16-18]. P-gp is an adenosine-50-triphosphate (ATP) dependent efflux drug transporter that is constitutively expressed in normal tissues that includes gastrointestinal epithelium, canalicular membrane of the liver, kidney [19, 20] and capillary endothelial cells in the central nervous system [21, 22]. Because of such tissue localized and its broad substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs [23, 24]. It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap with each other [25]. Dorababu et al. [26] reported that nifedipine belonged to a group of P-gp substrate. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote

presystemic drug metabolism, resulting in the limited absorption of drugs.

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 (atorvastatin, fluvastatin, pravastatin and simvastatin), possibly through the inhibition of CYP 3A4 and P-gp [27, 28]. But there are fewer reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [29, 30].

There are some reports on the effects of calcium channel antagonists on the pharmacokinetics of HMG-CoA reductase inhibitors. Calcium-channel blockers increased the plasma concentrations of some statins, possibly through the inhibition of CYP 3A4 and P-gp [31]. However, there are few reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of antihypertensive agents[29, 30, 32, 33]. Although clinically nifedipine and statins can be prescribed as a combination therapy for treatment of hypertension, pharmacokinetic interaction between a HMG-CoA reductase inhibitor and nifedipine in vivo has not been reported yet. Therefore, the present study aims to investigate the effect of HMG-CoA reductase inhibitors (atorvastatin, fluvastatin, pravastatin and simvastatin) on CYP3A4 activity, P-gp activity and the pharmacokinetics of nifedipine and its active metabolite, dehydronifedipine, after oral and intravenous administration in rats.

B. Materials and Methods

1. Materials

Nifedipine, dehydronifedipine, atorvastatin, fluvastatin, pravastatin, simvastatin and amlodipine [internal standard for the high-performance liquid chromatographic (HPLC) analysis of nifedipine] were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol, isooctane, methyl-*tert*-butyl ether (MTBE), analytical grade acetic acid and triethylamine (TEA) were products from Merck Co. (Darmstadt, Germany). Rhodamine was from Calbiochem (USA), the CYP inhibition assay kit was from GENTEST (Woburn, MA, US). Other chemicals were of reagent or HPLC grade.

Apparatus used in this study included an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a Waters^{TM} 2487 scanning UV detector (Waters Co., Milford, MA, USA), an 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 microcentrifuge (Hitachi Co., Tokyo, Japan).

2. Animal studies

All animal study protocols were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given free access to a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea) and tap water. Throughout the experiments, the animals were housed, four or five per cage, in laminar flow cages maintained at 22±2^{o}C, 50-60% relative humidity, under a 12 h light-dark cycle. The rats were acclimated under these conditions for at least 1 week. Each rat was fasted for at least 24 h prior to the experiment. The left femoral artery (for blood sampling) and left femoral vein (for drug administration in the intravenous study) were cannulated using a polyethylene tube (SP45; i.d., 0.58 mm, o.d., 0.96 mm; Natsume Seisakusho Company, Tokyo, Japan) while each rat was under light ether anesthesia.

3. Intravenous and oral administration of nifedipine

The rats were divided into eight groups (n=6, each): oral groups [10 mg/kg of nifedipine dissolved in distilled water (1.0 mL/kg)] without (control) or with 1.0 mg/kg of atorvastatin, fluvastatin, pravastatin and simvastatin (mixed in distilled water; total oral volume of 1.0 mL/kg), and intravenous groups (2.5 mg/kg of nifedipine; the same solution used: 0.9% NaCl-injectable solution; total injection volume of 1.0 mL/kg) without (control) or with 1.0 mg/kg of atorvastatin, fluvastatin, pravastatin and simvastatin. A feeding tube was used to administer nifedipine and HMG-CoA reductase inhibitors intragastrically.

Atorvastatin, fluvastatin, pravastatin and simvastatin was administered 30 min prior to oral administration of nifedipine. A blood sample (0.5-mL aliquot) was collected into heparinized tubes via the femoral artery at 0.017 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h for the intravenous study, and 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, and 24 h for the oral study. Whole blood (approximately 1.2 mL) collected from untreated rats was infused via the femoral artery at 0.75, 4, and 8 h, respectively, to replace blood loss due to blood sampling. The blood samples were centrifuged (13,000 rpm, 3 min), and a 200-μL aliquot of plasma samples was stored in the deep freezer at -40^{o}C until the HPLC analysis.

4. HPLC assay

The plasma concentrations of nifedipine were determined using an HPLC assay by a modification of the method reported by Grundy et al. [34]. Briefly, 50-μL of amlodipine (3 μg/mL), as the internal standard and 50-μL of 1.0 M sodium hydroxide were added to 0.2-mL of the plasma sample. It was then mixed for 3 s and 1-mL MTBE:isooctane (75:25, v/v) was added. The resultant mixure was vortex-mixed for 1 min and centrifuged at 3,000 rpm for 5 min. The organic layer (0.8 mL) was transferred into a clean test tube and evaporated under a gentle stream of nitrogen gas (no heat applied). The dried extract was reconstituted with 200 μl of mobile phase vortex-mixed for 1 min and aliquots of 160 μl were transferred to a clean autosampler vial. A 70-μL aliquot of the supernatant was injected into the HPLC system. The UV detector wavelength was set to 350 nm; and the column, a Nova-pack C_8 (100mm×8 mm I.D., 4 μm; Waters Co., Milford, MA, USA), was used at room temperature. A mixture of methanol:water (62:38, v/v, pH 4.5, adjusted with acetic acid, 320 μL TEA/1000 mL mixture was added) was used as the mobile phase at a flow rate of 1.0 mL/min. The retention times were: internal standard at 16.8 min, nifedipine at 8.2 min, and dehydronifedipine at 6.5 min (Fig.1-3). The detection limits of nifedipine and dehydronifedipine in rat plasma were all 5 ng/mL. The coefficients of variation for nifedipine and dehydronifedipine were all below 5.0%.

5. CYP inhibition assay

The inhibition assays on the human CYP3A4 enzyme activities was performed in multiwell plates using the CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [35]. Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates [50 mM 7-Benzyloxy-4-(trifluoromethyl) couamrin (7-BFC)] were incubated with or without test compounds in a reaction mix containing 1 pmol of P450 enzyme and the NADPH generating system (1.3 mM NADP, 3.54mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) 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 controls (1 μM

ketoconazole) were run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and results are expressed as the percent inhibition.

6. Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a reported method [36]. MCF-7/ADR cells, a doxorubicin-resistant human breast cancer cell line, were seeded on 24-well plates at a seeding density of 10^5 cells. MCF-7 cells are basically same to MCF-7/ADR cells. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to 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 of HMG-CoA reductase inhibitors (1, 3, 10, 30, 50 and 100 μM) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured at 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 ratio to control values. Verapamil (100 μM) was used as a positive control.

7. Pharmacokinetic analysis

Standard methods [37] were used to calculate the AUC [38] and $t_{1/2}$ using non-compartmental analysis (WinNonlin software version 4.1; Pharsight Corporation, Mountain View, CA, USA). The F was estimated by $AUC_{oral}/AUC_{i.v.} \times Dose_{i.v.}/Dose_{oral} \times 100$. The peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of nifedipine and dehydronifedipine were directly read from the experimental data.

8. Statistical analysis

A p-value <0.05 was deemed to be significant using a Social Package of Statistical Sciences (SPSS) posteriori analysis of variance (ANOVA) for the unpaired data and then individual differences among groups were determined using Duncan's multiple range test. All data are expressed as mean±SD except median (ranges) for T_{max}.

C. Results

1. Inhibition assay of CYP3A4 activity

The inhibitory effects of HMG-CoA reductase inhibitors on CYP3A4 activities are shown in Figure 4-8. Ketoconazole, atorvastatin, fluvastatin, pravastatin and simvastatin inhibited CYP3A4 activities with IC_{50} values of 0.1, 47.0, 5.4, 14.8 and 3.1 μM, respectively.

2. Rhodamine-123 retention assay

The effect of HMG-CoA reductase inhibitors on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells is shown in Figure 9-12. Accumulation of rhodamine-123 was reduced in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp.
In this study, the cell-based P-gp activity test using rhodamine-123 showed that fluvastatin and simvastatin (3-100 μM) significantly inhibited P-gp activity. Atorvastatin and pravastatin (30-100 μM) inhibited P-gp activity but not significantly .

3. Effects of atorvastatin, fluvastatin, pravastatin and simvastatin on the pharmacokinetics of nifedipine

Figure 13-16 show the mean plasma concentration–time profiles of nifedipine after oral administration (10 mg/kg) with or without HMG-CoA reductase inhibitors. Table 1-4 list the relevant pharmacokinetic parameters of nifedipine after oral administration. The AUC and C_{max} of nifedipine with fluvastatin (42.4 and 40.4%) were significantly ($p<0.05$) increased, respectively compared to those of control. The AUC and C_{max} of nifedipine with simvastatin (40.0, 31.7% in dose of 0.3 mg, 51.0 and 46.6% in dose of 1.0 mg) were significantly ($p<0.05$, $p<0.01$) increased, respectively compared to those of control. Consequently, the absolute bioavailability (AB) of nifedipine with fluvastatin was significantly ($p<0.05$) increased by 42.4% compared to that of control, and the absolute bioavailability (AB) of nifedipine with simvastatin was significantly ($p<0.05$, $p<0.01$) increased by 41.2 and 51.9%, respectively compared to that of control. The CL/F of nifedipine after oral administration with fluvastatin was significantly ($p<0.05$) decreased by 50.6% compared to that of control. The CL/F of nifedipine after oral administration with simvastatin was significantly ($p<0.05$, $p<0.01$) decreased by 46.9 and 55.7%, respectively compared to that of control. However, The AUC and C_{max} of nifedipine with atorvastatin and/or pravastatin were increased but these were not significant compared to those of control. The other pharmacokinetic parameters of nifedipine with atorvastatin and /or pravastatin were comparable to those of control.

4. Effect of atorvastatin, fluvastatin, pravastatin and simvastatin on the pharmacokinetics of metabolite,

dehydronifedipine

Figure 17-20 depict the mean plasma concentration–time profiles of dehydronifedipine after oral administration of nifedipine (10 mg/kg) without and with HMG-CoA reductase inhibitors. As listed in Table 2, the pharmacokinetic parameters of nifedipine with HMG-CoA reductase inhibitors were comparable to those of controls. The AUC of nifedipine with fluvastatin and simvastatin were significantly ($p<0.05$) increased by 21.9 and 27.5%, respectively compared to those of control. The metabolite-parent AUC ratio (MR) of nifedipine with fluvastatin and simvastatin were significantly ($p<0.05$) decreased by 16.0 and 18.4%, respectively compared to that of controls, suggesting that CYP3A subfamily-mediated metabolism of nifedipine in the intestine and/or in the liver were effectively inhibited by fluvastatin and simvastatin.

5. Effect of atorvastatin, fluvastatin, pravastatin and simvastatin on the pharmacokinetics of i.v.nifedipine

Mean arterial plasma concentration-time profiles of nifedipine following an intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence or absence of HMG-CoA-reductase inhibitor (1 mg/kg) are shown in Figure 21-24, the corresponding pharmacokinetic parameters are shown in Table 3.
The AUC of nifedipine was increased, but was not statistically significant compared to that in the control. The $t_{1/2}$ of nifedipine was also prolonged, but this increase was not significant. The pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of HMG-CoA reductase inhibitors in contrast to those of oral nifedipine. Accordingly, the enhanced oral bioavailability in the presence of HMG-CoA-reductase inhibitor, while there was no significant change in the pharmacokinetics of intravenous nifedipine, may be mainly due to inhibition of the CYP3A-mediated metabolism of nifedipine in the small intestine and/or in the liver, and to inhibition of the P-gp efflux transporter in the small intestine rather than renal elimination of nifedipine by HMG-CoA-reductase inhibitor.

D. Discussion

Based on the broad overlap in the substrate specificities as well as their co-localization in the small intestine, the primary site of absorption for orally administered drugs, cytochrome P450 (CYP) 3A4 and P-glycoprotein (P-gp), have been recognized as a concerted barrier to drug absorption [39, 40]. The prescription of more than one drug as a combination therapy is increasingly common in current medical practice. Cholesterol-lowering agents such as HMG-CoA reductase inhibitors could be co-administered with calcium channel blockers in the treatment of hypertension [41].

Considering that the drugs used in combination therapy often share the same metabolic pathways or cellular transport pathways, there exist high potential for pharmacokinetic as well as pharmacodynamic drug interactions between calcium channel antagonists and HMG-CoA reductase inhibitors. Indeed, some studies have reported that calcium-channel blockers increased the plasma concentrations of lovastatin or simvastatin [27, 28]. Similarly, as the dual substrates of CYP 3A and P-gp, nifedipine and HMG-CoA reductase inhibitor may undergo the same metabolic pathways and/or cellular transport pathways after co-administration. Therefore, HMG-CoA reductase inhibitor could affect the bioavailability or pharmacokinetics of nifedipine in rats.

In Figure 6-8, the inhibitory effects of atovastatin, lovastatin, pravastatin and simvastatin on CYP3A4 activity are shown and fluvastatin and simvastatin inhibited CYP3A4 activity with an IC_{50} value of 5.4 and 3.1 μM. In Figure 10 and 12, the concurrent use of fluvastatin (100 μM) and simvastatin (3-10 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner, it is suggested that fluvastatin and simvastatin significantly inhibited P-gp activity. These results were consistent with reports showing that lovastatin is an effective inhibitor of P-gp and CYP 3A transport [42, 43]. Some in-vitro and in-vivo studies have indicated that nifedipine is metabolized to dehydronifedipine mainly by CYP3A4 enzymes [11, 12]. Furthermore, Dorababu et al. [26] suggested that nifedipine is a substrate of P-gp. Considering that nifedipine is a substrate of both CYP enzymes and P-gp, fluvastatin and simvastatin, as a dual inhibitor of both CYP3A4 and P-gp, may significantly impact the pharmacokinetics and bioavailability of nifedipine.

CYP3A4 expressed in rat is similar and corresponding to the function of CYP3A4 in human [29, 30, 32, 33, 44]. As shown in Table 1-4, the presence of atorvastatin, fluvastatin, pravastatin and simvastatin significantly enhanced the $AUC_{0-\infty}$ and C_{max} of nifedipine in rats. Subsequently, the relative bioavailability (RB) of nifedipine was increased by 140 and 151% in the presence of fluvastatin and simvastatin (1.0 mg/kg), respectively. Those results were similar to reports by Chung et al and Hong et al. [29, 30] in that lovastatin significantly enhanced the $AUC_{0-\infty}$ and C_{max} of diltiazem and nicardipine, a substrate of both CYP enzymes and P-gp in rats, and by Yang et al. and Choi et al. [32, 33] in that HMG-CoA-reductase inhibitor significantly enhanced the AUC and C_{max} of verapamil and losartan in rats. Results of these studies were also consistent with the report that oral diallyl trisulfide (major organosulfur compounds derived from garlic) significantly increased the bioavailability of nifedipine by inhibition of CYP3A4 in

rats [45]. These results are consistent with the report by Kuroha et al. in that ketoconazole, a CYP3A4 inhibitor, significantly increased the $AUC_{0-\infty}$ and C_{max} of nifedipine [46].

The $AUC_{0-\infty}$ of dehydronifedipine were increased by the presence of 1.0 mg/kg of oral fluvastatin and simvastatin (Table 6 and 8), were statistically significant (21.9 and 27.5%; $p<0.05$), respectively. The metabolite-parent ratio (MR) in the presence of fluvastatin and simvastatin (1.0 mg/kg) were significantly ($p<0.05$) decreased, respectively compared to that of the control group (16.0 and 18.4%). Those results were similar to reports by Hong et al. Yang et al [30, 32] in that the metabolite-parent ratio (MR) of diltiazem and losartan in the presence of fluvastatin and simvastatin (1.0 mg/kg) were significantly ($p<0.05$) decreased, respectively compared to that of the control group.

After intravenous administration of nifedipine with HMG-CoA-reductase inhibitor, the AUC of nifedipine increased, but was not statistically significant (Table 9-12). The CL_t and $t_{1/2}$ values of nifedipine tend to decrease, but these were not statistically significant. This suggests that the effects of oral HMG-CoA-reductase inhibitor on the inhibition of hepatic metabolism of nifedipine via CYP3A4 were almost negligible. In contrast to those of oral nifedipine, the pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of HMG-CoA-reductase inhibitor.

Since the present study raised awareness of potential drug interactions by concomitant use of HMG-CoA-reductase inhibitor with nifedipine, this finding has to be further evaluated in clinical studies. The increased bioavailability of nifedipine in the presence of fluvastatin and simvastatin might be due to an inhibition of the P-gp-mediated efflux transporter and CYP 3A-mediated metabolism by HMG-CoA-reductase inhibitor.

E. Conclusion

The enhanced bioavailability of nifedipine after its oral administration with atorvastatin, fluvastatin, pravastatin and simvastatin could be mainly due to inhibition of both P-gp in the small intestine and CYP3A subfamily-mediated metabolism of nifedipine in the small intestine and/or in the liver and to the reduction of the CL/F of nifedipine by HMG-CoA-reductase inhibitor.

Present study raised awareness of potential drug interactions by concomitant use of nifedipine with HMG-CoA-reductase inhibitors.

If these results are confirmed in clinical trials, the nifedipine dosage regimen should be adjusted when nifedipine is coadministered with fluvastatin and/or simvastatin.

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Table 1. Mean (± SD) pharmacokinetic parameters of nifedipine after oral (10 mg/kg) administration with atorvastatin (0.3, 1 mg/kg) to rats (n = 6 each).

Parameter	Control	Atorvastatin	
		0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	5965 ± 1014	6569 ± 1049	7012 ± 1192
C_{max} (ng/ml)	1131 ± 203	1165 ± 209	1200 ± 216
T_{max} (h)	0.71 ±0.19	0.71 ± 0.19	0.71 ±0.19
$t_{1/2}$ (h)	9.7 ± 1.9	10.0 ± 1.9	10.0 ± 2.0
CL/F (ml/min/kg)	2.94 ± 0.69	2.41 ± 0.67	2.12 ± 0.65
AB(F) (%)	15.8 ±2.6	17.5 ± 2.9	18.6 ±3.1
RB (%)	100	110	117

AUC = Total area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; CL/F = total body clearance; AB(F) = absolute bioavailability; RB = relative bioavailability.

Statistically significant difference from control: * $p < 0.05$

Table 2. Mean (± SD) pharmacokinetic parameters of nifedipine after oral (10 mg/kg) administration with fluvastatin (0.3, 1 mg/kg) to rats (n = 6 each).

Parameter	Control	Fluvastatin	
		0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	5965 ± 1014	7277 ± 1359	8496 ± 1444*
C_{max} (ng/ml)	1131 ± 203	1382 ± 279	1588 ± 285*
T_{max} (h)	0.71 ±0.19	0.71 ±0.19	0.63±0.14
$t_{1/2}$ (h)	9.7 ± 1.9	10.1 ± 2.0	10.2± 2.0
CL/F (ml/min/kg)	2.94 ± 0.69	1.62 ± 0.69	1.45 ± 0.66*
AB(F) (%)	15.8 ±2.6	20.1 ± 3.7	22.5 ± 3.8*
RB (%)	100	122	142

AUC = Total area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; CL/F = total body clearance; AB(F) = absolute bioavailability; RB = relative bioavailability.

Statistically significant difference from control: * $p < 0.05$

Table 3. Mean (± SD) pharmacokinetic parameters of nifedipine after oral (10 mg/kg) administration with pravastatin (0.3, 1 mg/kg) to rats (n = 6 each).

Parameter	Control	Pravastatin	
		0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	5965 ± 1014	6800 ± 1362	7396 ± 1479
C_{max} (ng/ml)	1131 ± 203	1268 ± 250	1401 ± 293
T_{max} (h)	0.71 ±0.19	0.71 ±0.19	0.71 ±0.19
$t_{1/2}$ (h)	9.7 ± 1.9	9.8 ± 1.9	10.2 ± 2.0
CL/F (ml/min/kg)	2.94 ± 0.69	2.24 ± 0.68	1.86 ± 0.67*
AB(F) (%)	15.8 ±2.6	18.2 ± 3.5	19.8 ± 4.4
RB (%)	100	114	124

AUC = Total area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; CL/F = total body oral clearance; AB(F) = extent of absolute oral bioavailability; RB = relative bioavailability.

Statistically significant difference from control: * $p < 0.05$

Table 4. Mean (± SD) pharmacokinetic parameters of nifedipine after oral (10 mg/kg) administration with simvastatin (0.3, 1 mg/kg) to rats (n = 6 each).

Parameter	Control	Simvastatin	
		0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	5965 ± 1014	8351 ± 1650*	9008 ±1802**
C_{max} (ng/ml)	1131 ± 203	1490 ± 271*	1658 ± 294**
T_{max} (h)	0.71 ±0.19	0.63±0.15	0.63±0.15
$t_{1/2}$ (h)	9.7 ± 1.9	10.1 ± 2.0	10.4 ± 2.2
CL/F (ml/min/kg)	2.94 ± 0.69	1.56 ± 0.54*	1.30 ± 0.52**
AB(F) (%)	15.8 ±2.6	22.3 ± 3.9*	24.0 ± 4.1**
RB (%)	100	140	151

AUC = Total area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; CL/F = total body clearance; AB(F) = absolute bioavailability; RB = relative bioavailability.

Statistically significant difference from control: * $p < 0.05$, ** $p < 0.01$.

Table 5. Mean (± SD) Pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10 mg/kg) with atorvastatin (0.3, 1 mg/kg) to rats (n = 6 each)

		Atorvastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	2296 ± 459	2357 ± 462	2537 ± 482
C_{max} (ng/ml)	108 ± 19	112 ± 21	116 ± 22
T_{max} (h)	2.0 ± 0.63	2.0 ± 0.63	2.0 ± 0.63
$t_{1/2}$ (h)	15.7 ± 3.1	16.3 ± 3.2	16.6 ± 3.3
MR	0.38 ± 0.06	0.37 ± 0.06	0.36 ± 0.05

AUC = totoal area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; MR = metabolite-parent AUC ratio.

Table 6. Mean (± SD) Pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10 mg/kg) with fluvastatin (0.3, 1 mg/kg) to rats (n = 6 each)

		Fluvastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	2296 ± 459	2509 ± 502	2798 ± 532*
C_{max} (ng/ml)	108 ± 19	116 ± 22	124 ± 24
T_{max} (h)	2.0 ± 0.63	2.1 ± 0.76	2.1 ± 0.76
$t_{1/2}$ (h)	15.7 ± 3.1	16.7 ± 3.2	17.2 ± 3.3
MR	0.38 ± 0.06	0.34 ± 0.05	0.32 ± 0.03*

AUC = totoal area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; MR = metabolite-parent AUC ratio.

Statistically significant difference from control: $^{*}p < 0.05$.

Table 7. Mean (± SD) Pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10 mg/kg) with pravastatin (0.3, 1 mg/kg) to rats (n = 6 each)

		Pravastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	2296 ± 459	2469± 497	2670 ± 533
C_{max} (ng/ml)	108 ± 19	114 ± 21	120 ± 23
T_{max} (h)	2.0 ± 0.63	2.0 ± 0.63	2.0 ± 0.63
$t_{1/2}$ (h)	15.7 ± 3.1	16.3 ± 3.2	17.0 ± 3.3
MR	0.38 ± 0.06	0.37 ± 0.06	0.35 ± 0.05

AUC = totoal area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; MR = metabolite-parent AUC ratio.

Table 8. Mean (± SD) Pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10 mg/kg) with simvastatin (0.3, 1 mg/kg) to rats (n = 6 each)

		Simvastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	2296 ± 459	2741 ± 559	2927 ± 584*
C_{max} (ng/ml)	108 ± 19	119 ± 22	130 ± 25
T_{max} (h)	2.0 ± 0.63	2.0 ± 0.63	2.1 ± 0.76
$t_{1/2}$ (h)	15.7 ± 3.1	16.7 ± 3.2	17.4 ± 3.4
MR	0.38 ± 0.06	0.33 ± 0.06	0.31 ± 0.04*

AUC = totoal area under the plasma concentration–time curve from time zero to infinity; C_{max} = peak plasma concentration; T_{max} = time to reach the peak plasma concentration; $t_{1/2}$ = terminal half-life; MR = metabolite-parent AUC ratio.

Statistically significant difference from control: $^{*}p < 0.05$.

Table 9. Mean (±SD) pharmacokinetic parameters of nifedipine after the intravenous administration (2.5 mg/kg) to rats with atovastatin (0.3, 1 mg/kg; n = 6 each)

		Atorvastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	9443 ± 1856	9725 ± 1889	9915 ± 1903
CL_t (mL/hr/kg)	4.3 ± 0.8	4.2 ± 0.8	4.1 ± 0.8
$t_{1/2}$ (h)	8.9 ± 1.8	9.0 ± 1.8	9.1± 1.9
RB (%)	100	103	105

$AUC_{0-\infty}$ = Area under the plasma concentration-time curve from 0 h to infinity; CL_t = total body clearance; $t_{1/2}$ = terminal half-life; RB = relative bioavailability

Table 10. Mean (±SD) pharmacokinetic parameters of nifedipine after the intravenous administration (2.5 mg/kg) to rats with Fluvastatin (0.3, 1 mg/kg; n = 6 each)

		Fluvastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	9443 ± 1856	9943 ± 1903	10293 ± 1995
CL_t (mL/hr/kg)	4.3 ± 0.8	4.2 ± 0.8	3.9 ± 0.7
$t_{1/2}$ (h)	8.9 ± 1.8	9.2 ± 1.8	9.4 ± 2.1
RB (%)	100	105	109

$AUC_{0-\infty}$ = Area under the plasma concentration-time curve from 0 h to infinity; CL_t = total body clearance; $t_{1/2}$ = terminal half-life; RB = relative bioavailability

Table 11. Mean (±SD) pharmacokinetic parameters of nifedipine after the intravenous administration (2.5 mg/kg) to rats with pravastatin (0.3, 1 mg/kg; n = 6 each)

		Pravastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	9443 ± 1856	9820 ± 1903	10121 ± 1934
CL_t (mL/hr/kg)	4.3 ± 0.8	4.2 ± 0.8	4.0 ± 0.7
$t_{1/2}$ (h)	8.9 ± 1.8	9.2 ± 1.8	9.3 ± 1.9
RB (%)	100	104	107

$AUC_{0-\infty}$ = Area under the plasma concentration-time curve from 0 h to infinity; CL_t = total body clearance; $t_{1/2}$ = terminal half-life; RB = relative bioavailability

Table 12. Mean (±SD) pharmacokinetic parameters of nifedipine after the intravenous administration (2.5 mg/kg) to rats with simvastatin (0.3, 1 mg/kg; n = 6 each)

		Simvastatin	
Parameter	Control	0.3 mg/kg	1 mg/kg
AUC (ng·h/ml)	9443 ± 1886	10104 ± 1914	10482 ± 2008
CL_t (mL/hr/kg)	4.3 ± 0.8	4.1 ± 0.8	3.8 ± 0.7
$t_{1/2}$ (h)	8.9 ± 1.8	9.3 ± 1.9	9.5 ± 2.1
RB (%)	100	107	111

$AUC_{0-\infty}$ = Area under the plasma concentration-time curve from 0 h to infinity; CL_t = total body clearance; $t_{1/2}$ = terminal half-life; RB = relative bioavailability

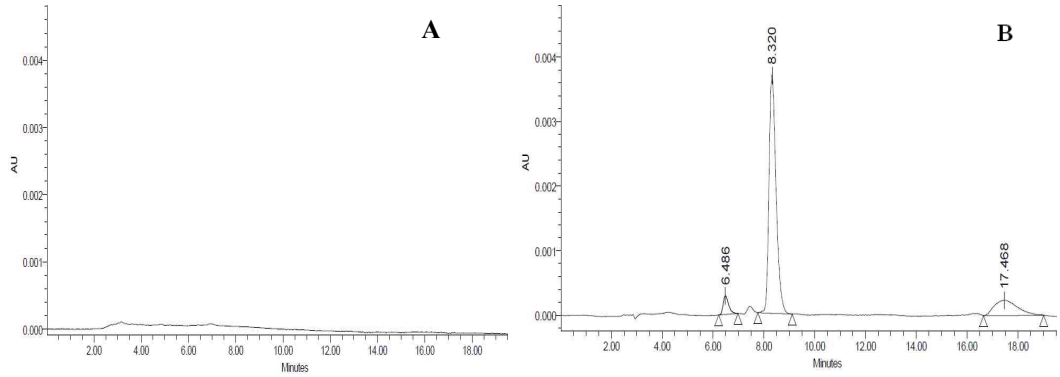

Figure 1. HPLC chromatograms of the rat blank plasma (A) and the plasma spiked with nifedipine (8.320 min), dehydronifedipine (6.486 min) and amlodipine (internal standard; 17.468 min) (B).

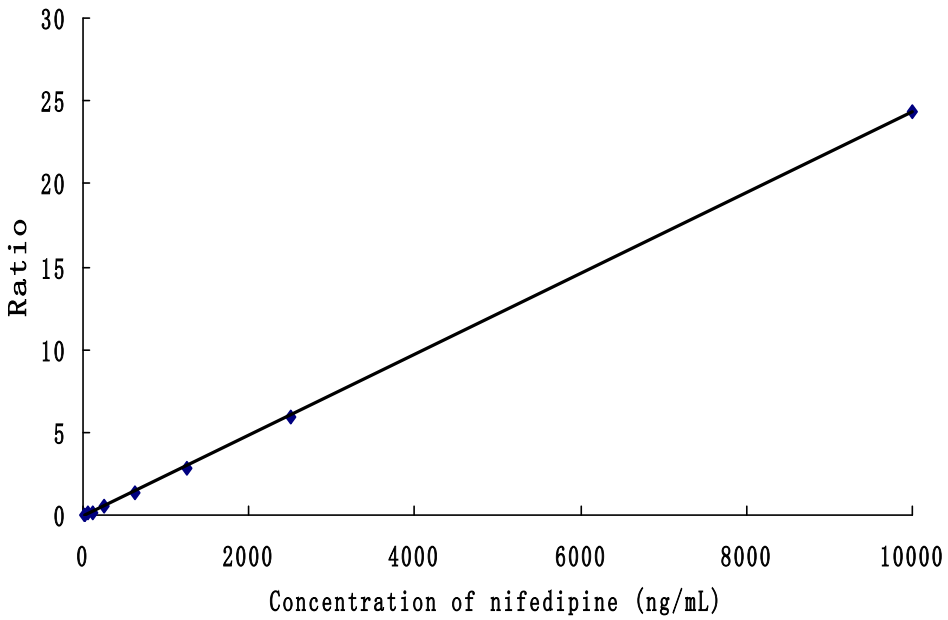

Figure 2. A calibration curve of nifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was y=0.0024x - 0.0983, where “y” is the peak area ratio of nifedipine to amlodipine and “x” is the concentration of nifedipine.

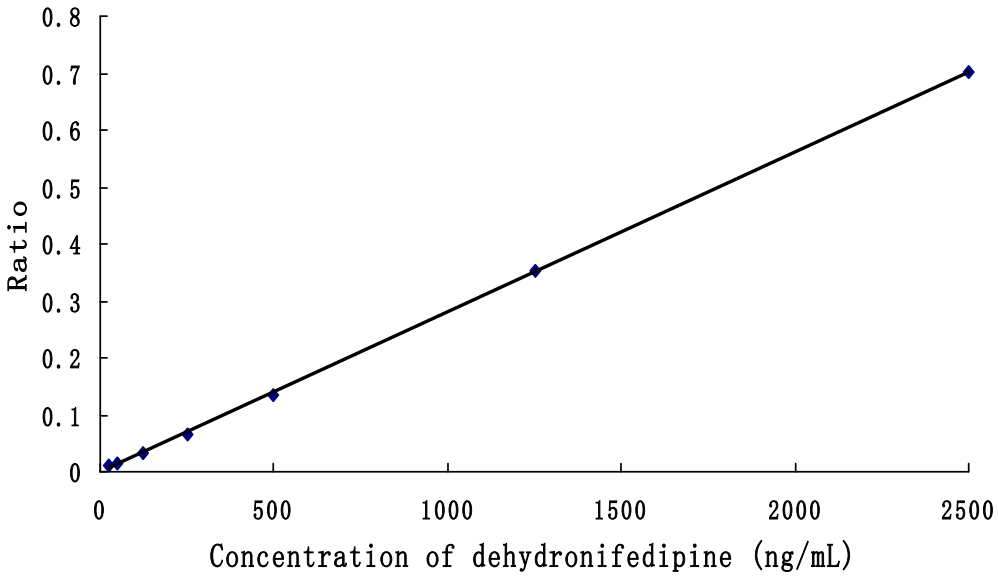

Figure 3. A calibration curve of_dehydronifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was y=0.0003x - 0.0014, where “y” is the peak area ratio of dehydronifedipine to amlodipine and “x” is the concentration of dehydronifedipine.

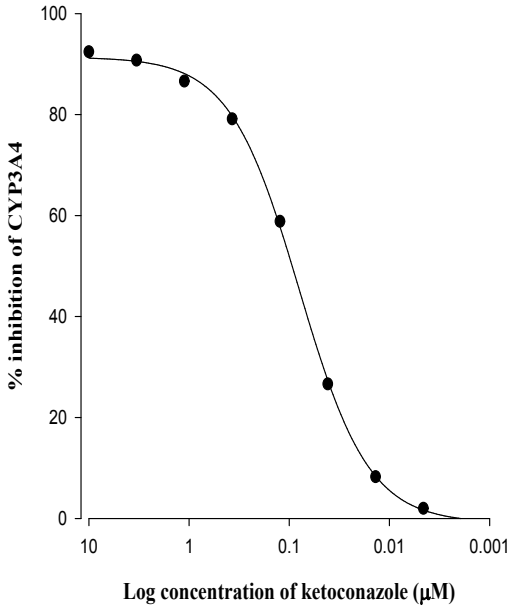

Figure 4. Inhibitory effects of ketoconazole on CYP3A4 activity. Results are expressed as the percent inhibition.

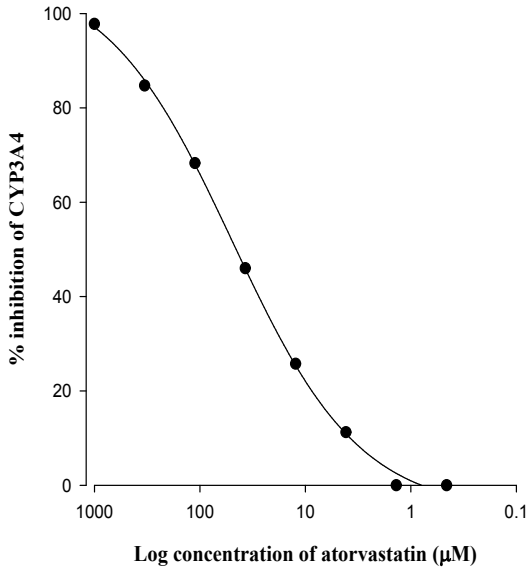

Figure 5. Inhibitory effects of atorvastatin on CYP3A4 activity. Results are expressed as the percent inhibition.

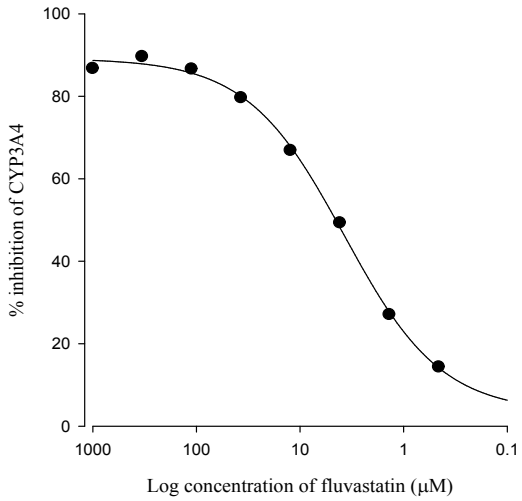

Figure 6. Inhibitory effects of fluvastatin on CYP3A4 activity. Results are expressed as the percent inhibition.

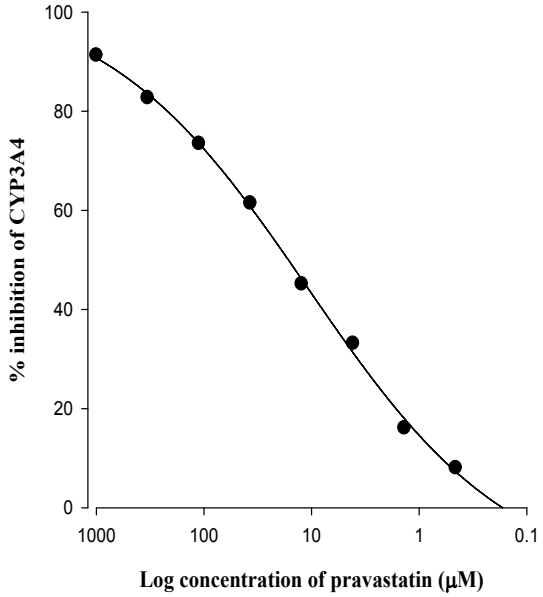

Figure 7. Inhibitory effects of pravastatin on CYP3A4 activity. Results are expressed as the percent inhibition.

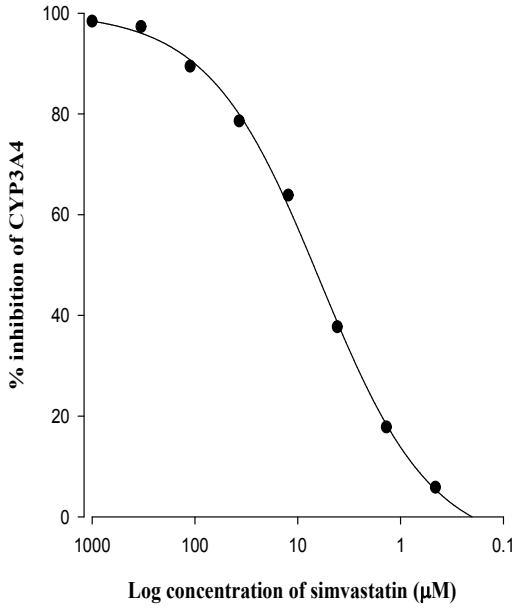

Figure 8. Inhibitory effects of simvastatin on CYP3A4 activity. Results are expressed as the percent inhibition.

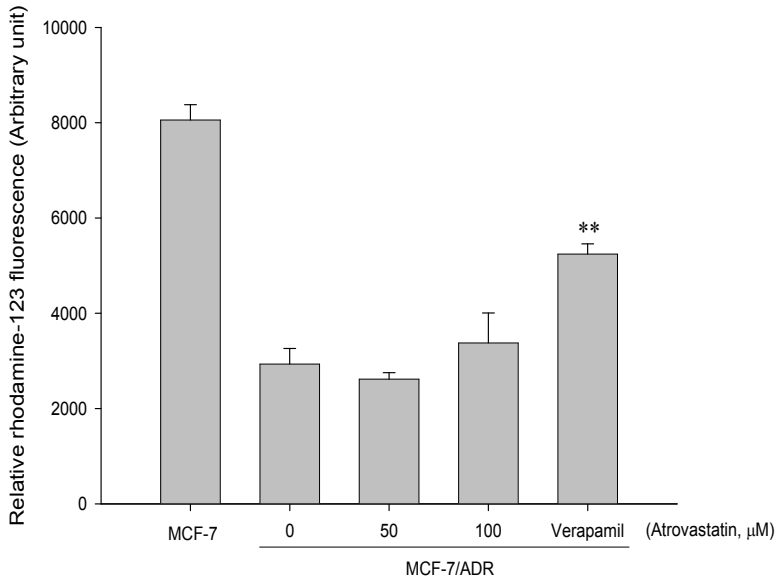

Figure 9. The effect of atorvastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD of 6 separate samples (* $p<0.05$).

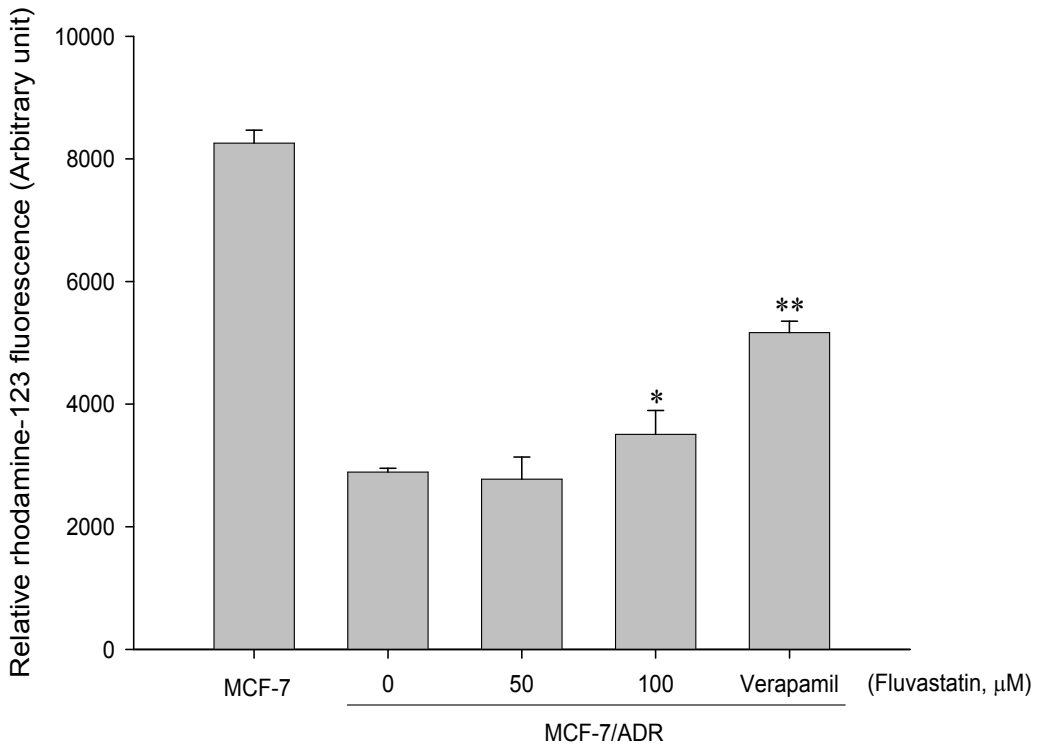

Figure 10. The effect of fluvastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD of 6 separate samples (* $p<0.05$).

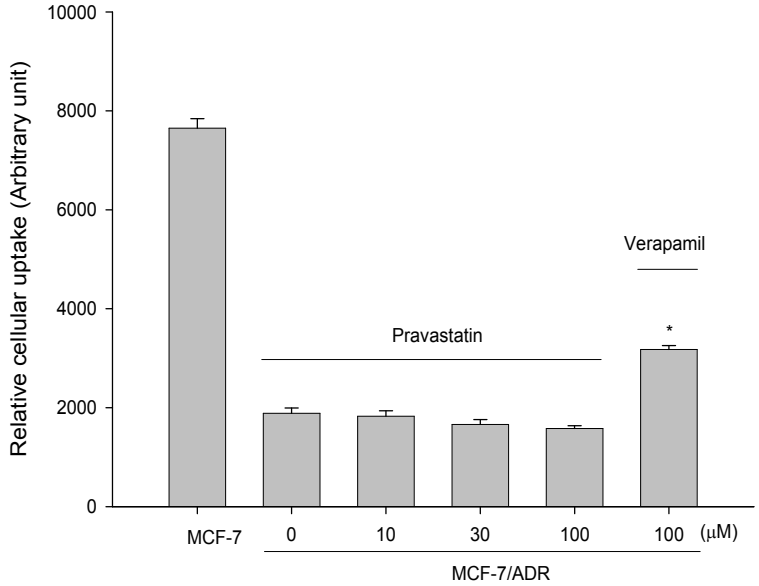

Figure 11. The effect of pravastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD of 6 separate samples (* $p<0.05$).

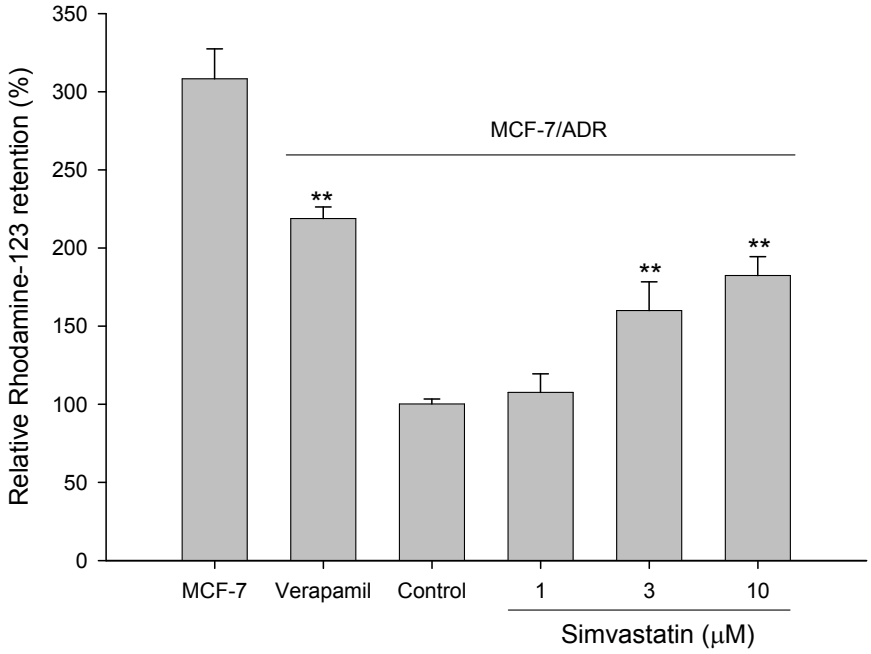

Figure 12. The effect of simvastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD of 6 separate samples (** $p<0.05$).

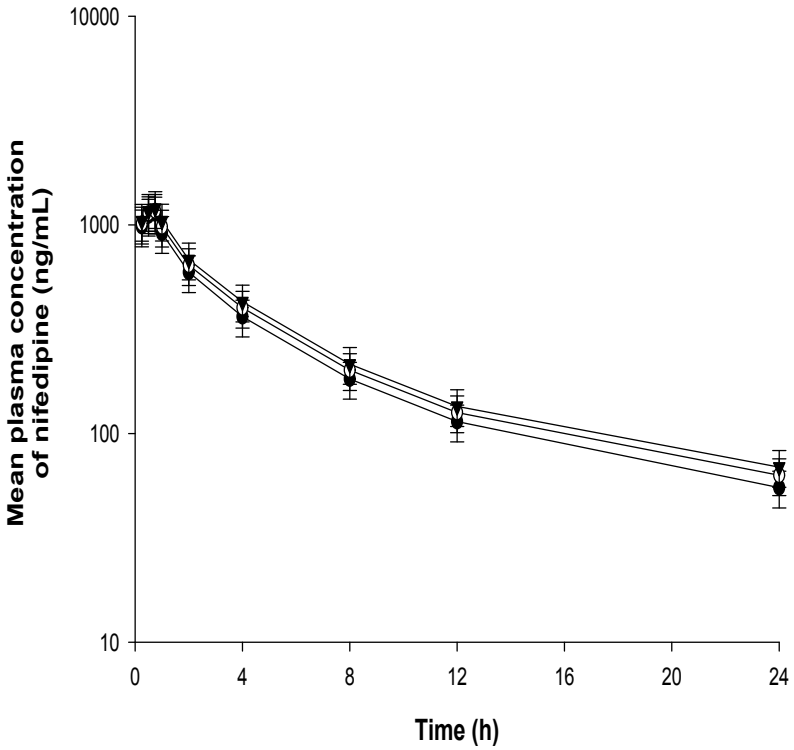

Figure 13. Mean plasma concentration-time profiles of nifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with atorvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg atorvastatin; ▼ = with of 1 mg/kg atorvastatin.

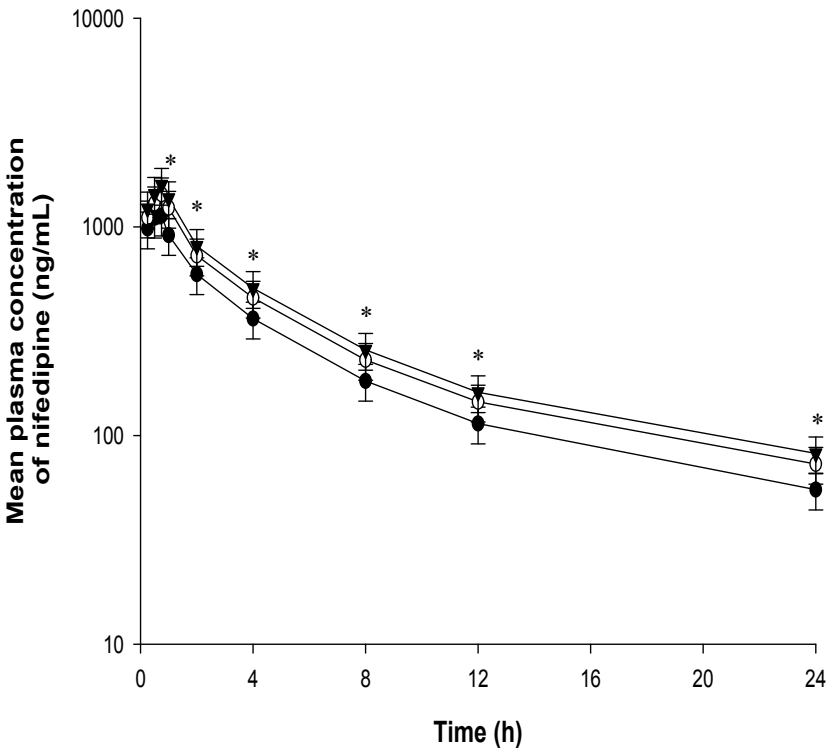

Figure 14. Mean plasma concentration-time profiles of nifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with fluvastatin (0.3, 1.0 mg/kg; n = 6, each). Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg fluvastatin; ▼ = with of 1.0 mg/kg fluvastatin (* $p<0.05$).

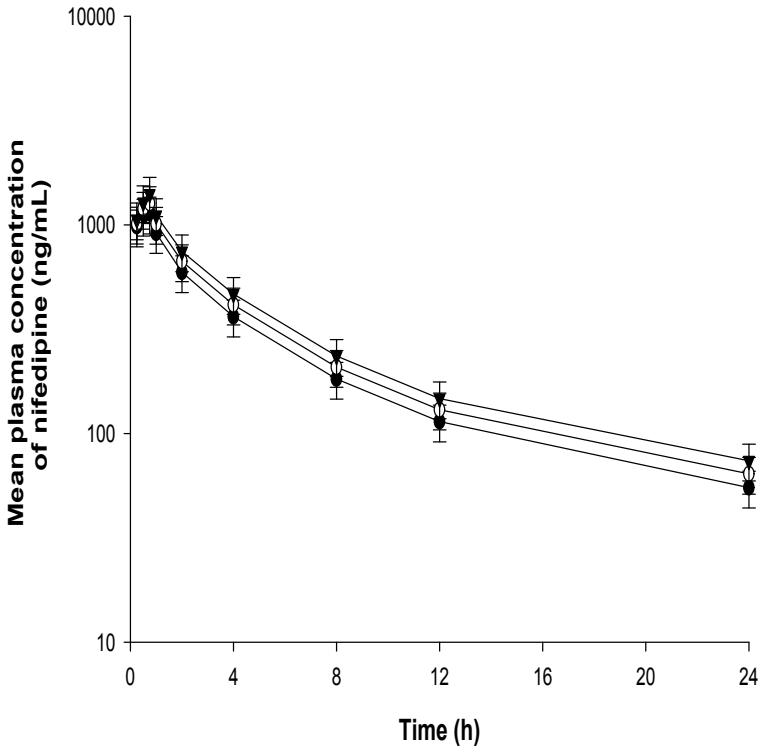

Figure 15. Mean plasma concentration-time profiles of nifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with pravastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg pravastatin; ▼ = with of 1 mg/kg pravastatin.

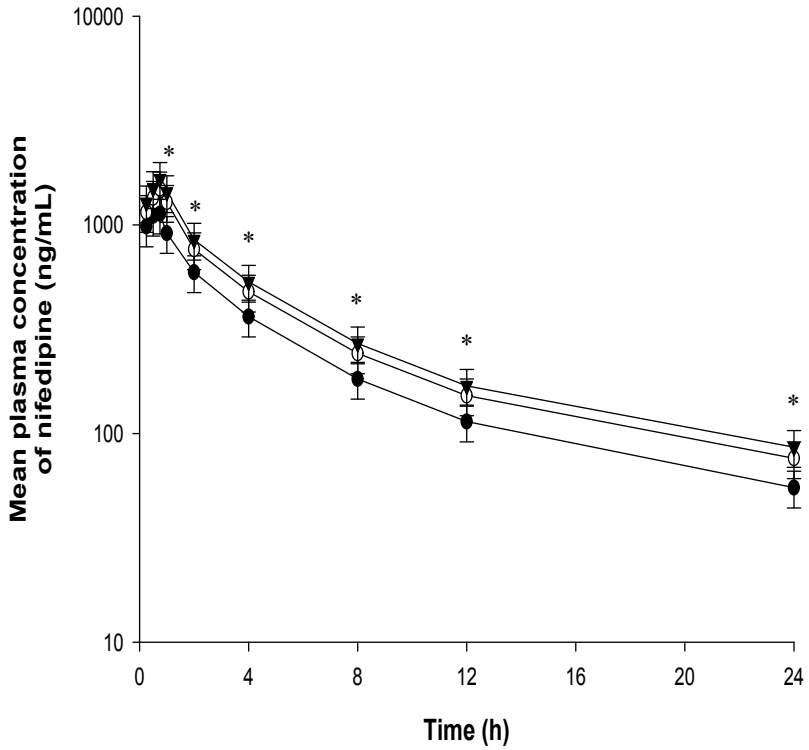

Figure 16. Mean plasma concentration-time profiles of nifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with simvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg simvastatin; ▼ = with of 1 mg/kg simvastatin (* $p<0.05$).

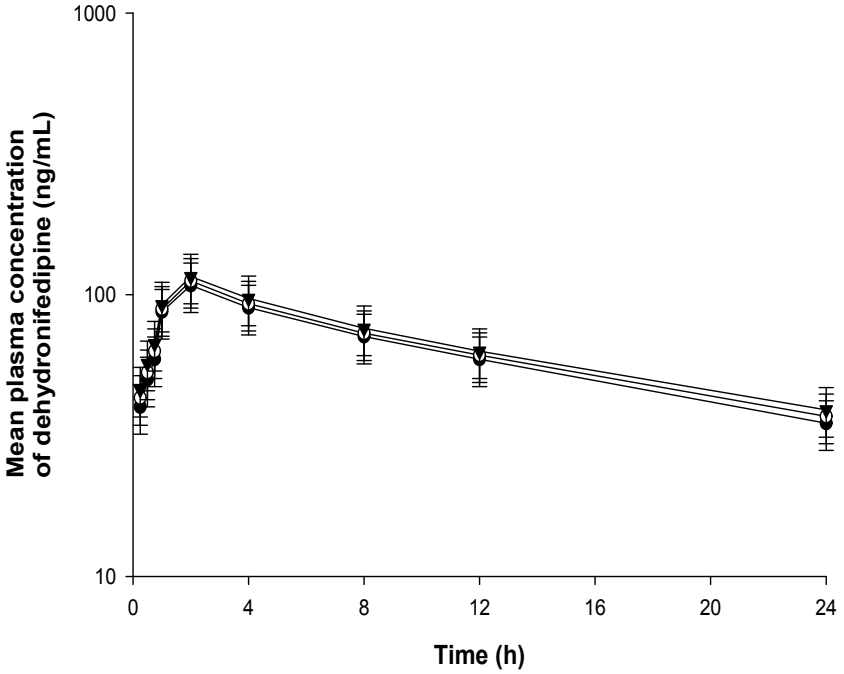

Figure 17. Mean plasma concentration-time profiles of dehydronifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with atorvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg atorvastatin; ▼ = with of 1 mg/kg atorvastatin.

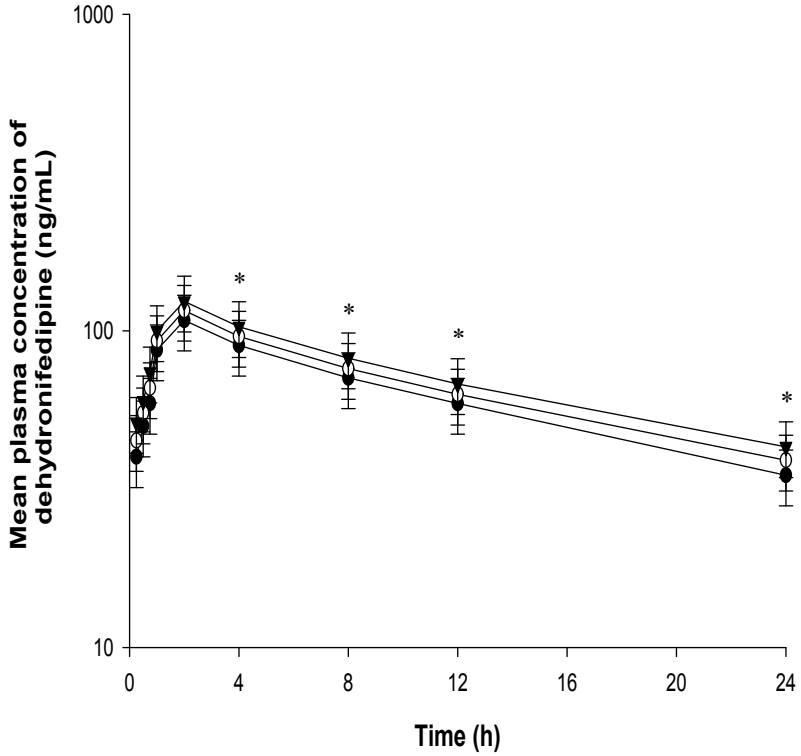

Figure 18. Mean plasma concentration-time profiles of dehydronifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with fluvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg fluvastatin; ▼ = with of 1 mg/kg fluvastatin (* $p<0.05$).

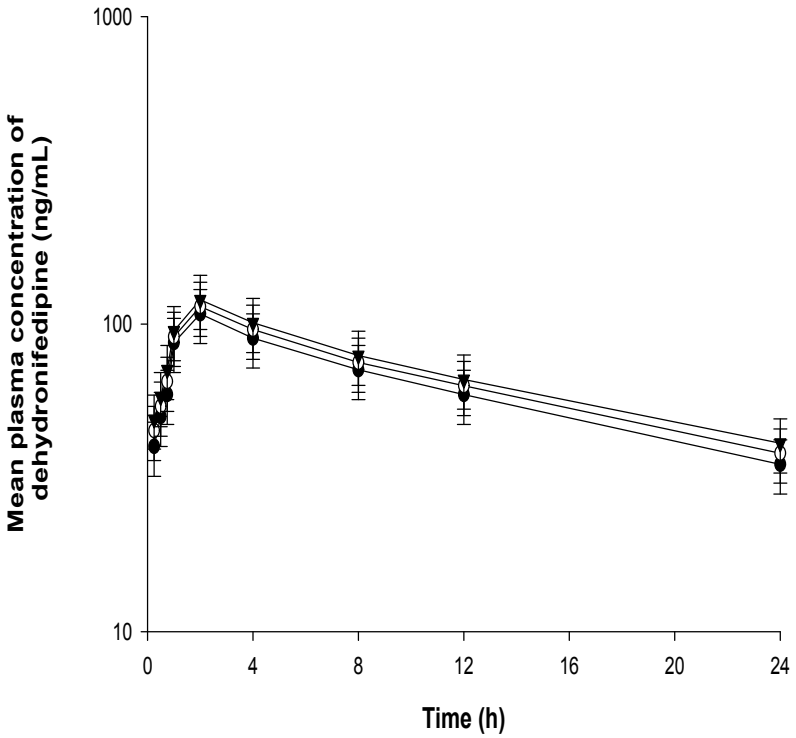

Figure 19. Mean plasma concentration-time profiles of dehydronifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with pravastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg pravastatin; ▼ = with of 1 mg/kg pravastatin.

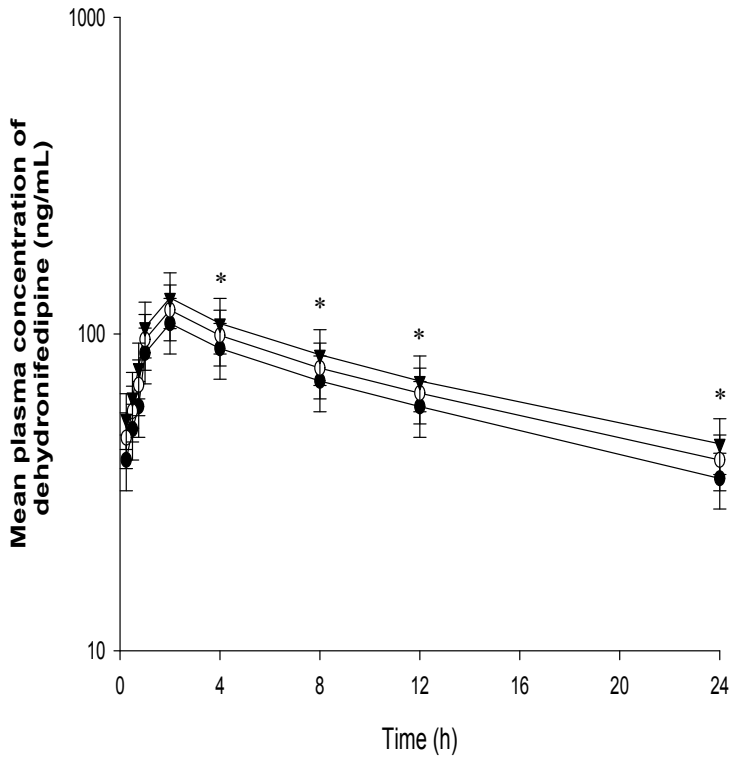

Figure 20. Mean plasma concentration-time profiles of dehydronifedipine after oral (10 mg/kg) administration of nifedipine to rats without and with simvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; Bars represent the standard deviation; ● =oral administration of nifedipine (10 mg/kg; control); ○ = with of 0.3 mg/kg simvastatin; ▼ = with of 1 mg/kg simvastatin (* $p<0.05$).

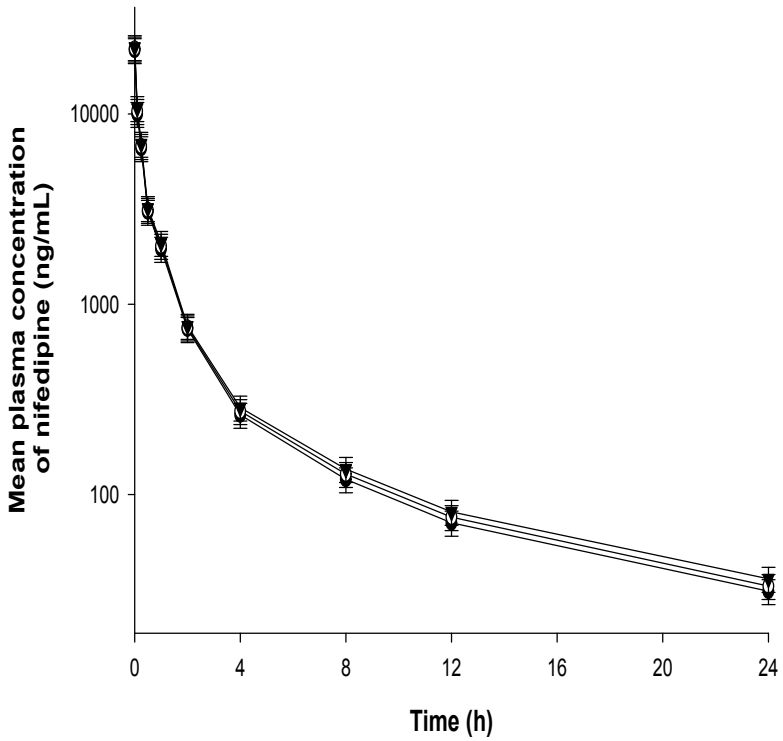

Figure 21. Mean plasma concentration-time profiles of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine to rats without and with atorvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● = intravenous administration of nifedipine (2.5 mg/kg; control); ○ = with of 0.3 mg/kg atorvastatin; ▼ = with of 1 mg/kg atorvastatin.

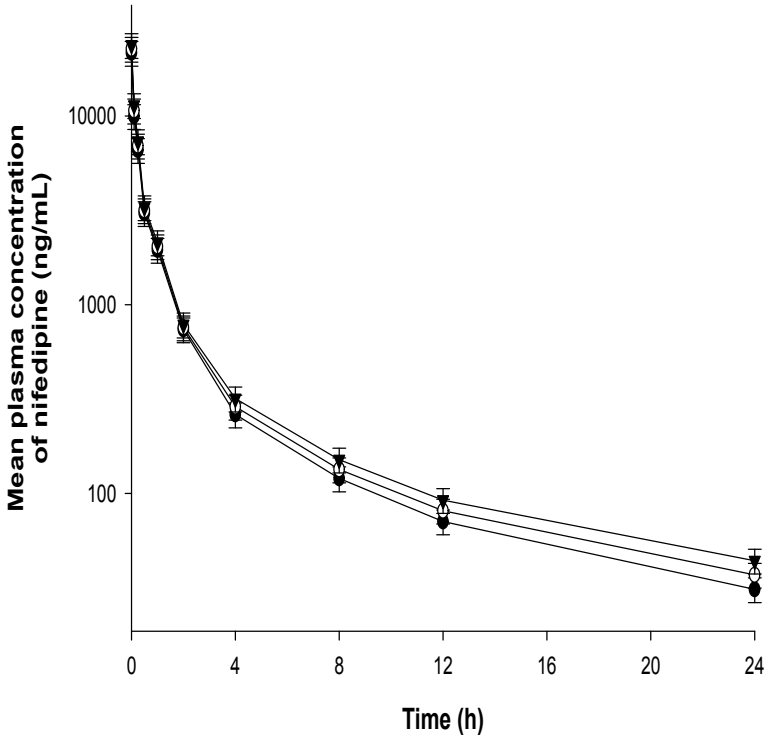

Figure 22. Mean plasma concentration-time profiles of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine to rats without and with fluvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● = intravenous administration of nifedipine (2.5 mg/kg; control); ○ = with of 0.3 mg/kg fluvastatin; ▼ = with of 1 mg/kg fluvastatin.

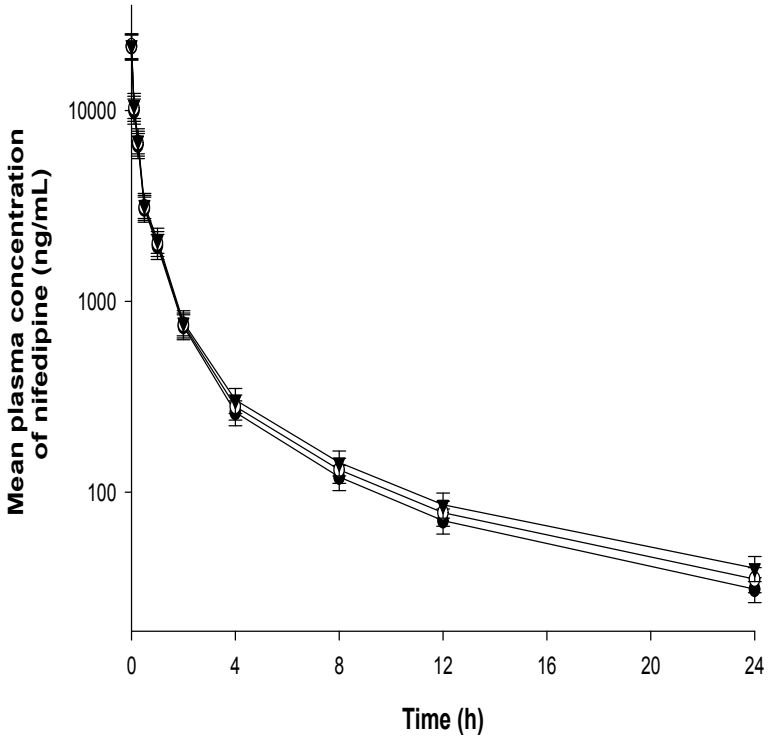

Figure 23. Mean plasma concentration-time profiles of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine to rats without and with pravastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● = intravenous administration of nifedipine (2.5 mg/kg; control); ○ = with of 0.3 mg/kg pravastatin; ▼ = with of 1 mg/kg pravastatin.

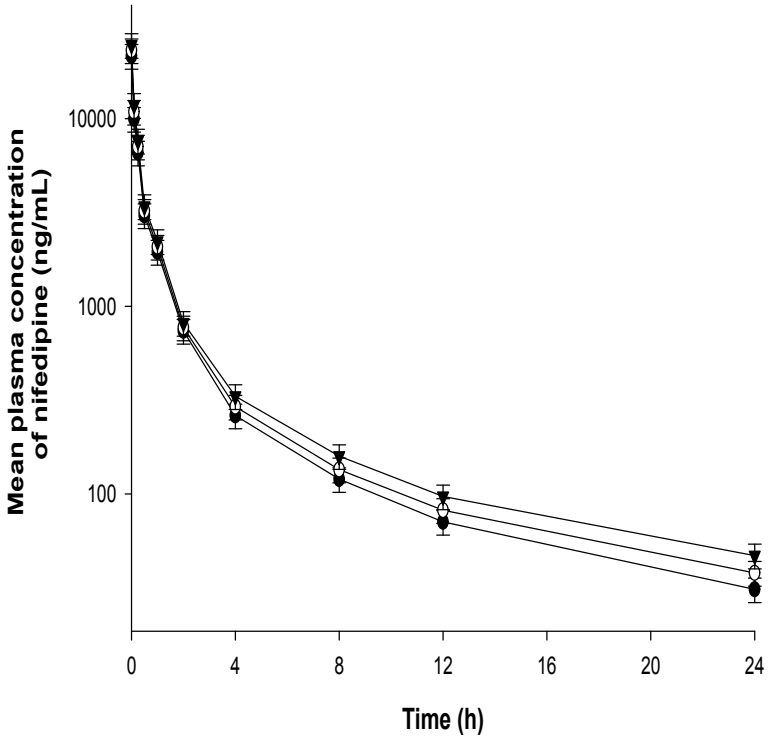

Figure 24. Mean plasma concentration-time profiles of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine to rats without and with simvastatin (0.3, 1 mg/kg; n = 6, each). Bars represent the standard deviation; ● = intravenous administration of nifedipine (2.5 mg/kg; control); ○ = with of 0.3 mg/kg simvastatin; ▼ = with of 1 mg/kg simvastatin.

ABSTRACT

Effects of HMG-CoA reductase inhibitors on the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats

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College of Medicine

Graduate School of Chosun University

The present study was designed to investigate the effects of HMG-CoA reductase inhibitors (atorvastatin, fluvastatin, pravastatin, simvastatin) on the pharmacokinetics of nifedipine and its active metabolite, dehydronifedipine in rats. Pharmacokinetic parameters of nifedipine and dehydronifedipine in rats were determined after oral and intravenous administration of nifedipine without and with HMG-CoA reductase inhibitors (0.3 or 1.0 mg/kg). The effect of HMG-CoA reductase inhibitors on p-glycoprotein (P-gp) and CYP3A4 activity were also evaluated. Atorvastatin, fluvastatin, pravastatin and simvastatin inhibited CYP3A4 activities with IC_{50} values of 47.0, 5.4, 14.8 and 3.1 μM, respectively. Simvastatin and fluvastatin (3-10, 100 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner, suggesting that P-gp activity was inhibited by fluvastatin and simvastatin.

The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of nifedipine were significantly ($p<0.05$) increased respectively by fluvastatin (1.0 mg/kg) compared to those of control. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of nifedipine were significantly ($p<0.05$, $p<0.01$) increased respectively by simvastatin (0.3 and 1.0 mg/kg) compared to those of control. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of nifedipine were increased respectively by atovastatin and pravastatin compared to those of control, but these were not singnificant. All pharmacokinetic parameters of nifedipine were not affected by low dosage of HMG-CoA reductase inhibitors (0.3 mg/kg).

The total body clearance (CL/F) of nifedipine after oral administration with fluvastatin (1.0 mg/kg) was significantly decreased (by 50.6%) compared to that of control. The total body clearance (CL/F) of nifedipine after oral administration with simvastatin (0.3 and 1.0 mg/kg) was significantly decreased (by 46.9 and 55.7%) compared to that of control.

Consequently, the absolute bioavailability (AB) of nifedipine after oral administration with fluvastatin was significantly increased (by 42.4%) compared to that of control. The absolute bioavailability (AB) of nifedipine after oral administration with simvastatin was significantly increased (by 41.1 and 51.9%) compared to that of control.

The absolute bioavailability (AB) of nifedipine after oral administration with atovastatin and pravastatin was increased compared to that of control, but this was not significant.

The relative bioavailability (RB) of nifedipine was 1.11-to 1.51-fold greater than that of the control by HMG-CoA reductase inhibitors.

The area under the plasma concentration-time curve ($AUC_{0-\infty}$) of dehydronifedipine were significantly ($p<0.05$) increased respectively by fluvastatin and simvastatin (1.0 mg/kg) compared to those of control.

The metabolite-parent AUC ratio (MR) of nifedipine with fluvastatin and simvastatin was significantly decreased respectively by 16.0 and 18.4%, suggesting that metabolism of nifedipine in the small intestine and/or liver was inhibited by fluvastatin 1.0 mg/kg) and simvastatin (0.3 and 1.0 mg/kg).

After intravenous administration of nifedipine with HMG-CoA-reductase inhibitor, the AUC of nifedipine increased, but was not statistically significant. The CL_t and $t_{1/2}$ values of nifedipine tend to decrease, but was not statistically significant. This suggests that the effects of HMG-CoA-reductase inhibitor on the inhibition of hepatic metabolism of nifedipine via CYP3A4 were almost negligible.

In conclusion, the enhanced bioavailability of nifedipine might be mainly due to inhibition of P-gp in the small intestine and CYP3A subfamily-mediated metabolism of nifedipine in the small intestine and/or liver and to reduction of the CL/F of nifedipine by fluvastatin and simvastatin.

Since the present study raised awareness of potential drug interactions by concomitant use of nifedipine with HMG-CoA-reductase inhibitors, this result has to be further evaluated for dosage regimen of nifedipine in clinical studies.

Keywords: Nifedipine; dehydronifedipine; HMG-CoA reductase inhibitors; pharmacokinetics; bioavailability; $AUC_{0-\infty}$; C_{max} ; total body clearance; P-gp; CYP3A subfamily; rats

Acknowledgements

새로운 배움과 학위를 취득하며 무언가를 이루었다는 성취감에 마음이 뿌듯하며 이 모든 것을 이루는데 밑거름이 되게 하신 모든 분들께 감사의 말을 전하고자 합니다.

좋은 연구 테마를 주시고 물심양면으로 격려와 조언으로 학위의 꿈을 주신 최동현 교수님께 머리숙여 감사를 드립니다.

항상 격려와 조언을 아끼지 않으신 순환기 내과 홍순표 교수님, 장경식 교수님, 고영엽 교수님과 정중화 교수님께 깊이 감사를 드립니다.

부족한 저의 학위논문을 지적 및 보완하여 심사하여 주신 김동민 교수님과 신병철 교수님께 감사를 드립니다.

학위논문을 실험할 때 도와주신 약학대학 최준식 교수님과 이 성 선생님께도 진심으로 감사를 드립니다.

항상 기도해 주시고 염려해 주신 부모님께 한없는 감사를 드립니다.

박사학위를 가진 의사로써 내이웃과 환자들에게 봉사하고, 더욱 더 매진하여 세상의 빛과 소금이 되어 하나님께 영광을 돌리는 의사가 되고자 노력 하겠읍니다.

저작물 이용 허락서

학 과	의학과	학 번		과 정	박사
성 명	한 글 :	한 문 :		영 문 :	
주 소	광주시				
연락처					
논문제목	한글:. 영문:				

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 ( )

2013 년 12 월

저작자: (서명 또는 인)

조선대학교 총장 귀하