2009 년 8 월

박사학위논문

DRUG INTERACTION BETWEEN SIMVASTATIN OR LOVASTATIN AND DILTIAZEM IN RATS

조선대학교 대학원

의 학 과

최 동 현

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흰쥐에서 심바스타틴 및 로바스타틴과 딜티아젬과의 약물상호작용

2009 년 8 월 25 일

조선대학교 대학원

의학과

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지도교수 홍 순 표

이 논문을 의학박사학위신청 논문으로 제출함.

2009 년 4 월

조선대학교 대학원

의학과

최 동 현

최동현의 박사학위논문을 인준함

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2009 년 6 월

조선대학교 대학원

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Drug Interaction between Simvastatin or Lovastatin with Diltiazem in Rats

Dong-Hyun Choi
Advisor: Prof. Soon-Pyo Hong, Ph.D.
Department of Medicine,
Graduate School Chosun University

HMG-CoA reductase inhibitors, statins and diltiazem, calcium channel blocker, could be prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. The purpose of this study was to investigate the possible effects of HMG-CoA reductase inhibitor, lovastatin and simvastatin, on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats.

The pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined after orall administration of diltiazem (12 mg/kg) to rats in the presence and absence of lovastatin (0.3 and 1.0 mg/kg) or simvastatin (0.3 and 1.0 mg/kg).

Compared to the control (diltiazem alone), lovastatin (1.0 mg/kg) or simvastatin (0.3 and 1.0 mg/kg) significantly altered the pharmacokinetic parameters of diltiazem. The area under the plasma concentration-time curve (AUC) and the peak plasma concentration (C_{max}) of diltiazem were significantly increased in the presence of lovastatin ($P < 0.05$, 1.0 mg/kg) or simvastatin ($P < 0.05$, 0.3 and 1.0 mg/kg), respectively. Consequently, the absolute bioavailability

of diltiazem in the presence of lovastatin (1.0 mg/kg) or simvastatin (0.3 and 1.0 mg/kg) were significantly higher ($P < 0.05$) than that of the control group, respectively.

Lovastatin (1.0 mg/kg) or simvastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the AUC of desacetyldiltiazem, respectively. Moreover, the metabolite-parent AUC ratio (MR) in the presence of lovastatin (1.0 mg/kg) or simvastatin (1.0 mg/kg) were significantly ($P < 0.05$) decreased compared to that of the control group, respectively. Lovastatin or simvastatin significantly reduced rhodamine 123 efflux via P-gp in MCF-7/ADR cell overexpressing p-gp. Simvastatin is more effective than lovastatin in inhibitory effect of P-gp.

In conclusion, the enhanced oral bioavailability of diltiazem by lovastatin or simvastatin may result from decreased P-gp-mediated efflux in small intestine and inhibition of CYP 3A subfamily metabolism in small intestine or in the liver. Based on these results, if these results would be confirmed in the patients with cardiovascular diseases, the dosage of diltiazem should be readjusted when diltiazem is used concomitantly with lovastatin or simvastatin.

Key words: Calcium channel blocker-Diltiazem, Desacetyldiltiazem, Statins-Lovastatin, Simvastatin, CYP3A, P-gp, Pharmacokinetics, Bioavailability, Rats

국 문 초 록

흰쥐에서 심바스타틴 및 로바스타틴과 딜티아젬과의 약물 상호작용

최 동 현

지 도 교 수: 홍 순 표

조선대학교대학원 의학과

HMG-CoA 환원효소 억제제와 딜티아젬의 병용투여가 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 있으므로 이에 대한 상호작용을 알아보고자, 흰쥐에 딜티아젬 (12 mg/kg) 과 로바스타틴 (0.3, 1.0 mg/kg) 또는 심바스타틴 (0.3, 1.0 mg/kg) 을 병용 경구투여한 후 딜티아젬 및 그 활성대사체인 데스아세틸딜티아젬의 약물동태학적 변수들을 대조군과 비교 검토하였다.

로바스타틴 또는 심바스타틴과 병용 투여시 딜티아젬의 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 로바스타틴 (1.0 mg/kg) 또는 심바스타틴 (0.3, 1.0 mg/kg) 과 병용투여군에서 딜티아젬의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max}) 는 각각 유의성 ($P < 0.05$) 있게 증가되었으며, 절대적생체이용률 (AB)도 대조군에 비해 각각 유의성 ($P < 0.05$) 있게 증가되었다.

아울러 로바스타틴 (1.0 mg/kg) 또는 심바스타틴 (1.0 mg/kg) 과 딜티아젬을 병용투여한군에서 대조군에 비해 활성대사체인 데스아세틸딜티아젬의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 ($P < 0.05$) 있게 증가되었다. 그리고 로바스타틴 또는 심바스타틴은 딜티아젬의

대사율(MR) 을 각각 유의성 있게 감소시켰다.

심바스타틴이 로바스타틴 보다 P-gp 억제효과가 더 컸다. 본 연구에서 고지혈증 치료제인 로바스타틴 또는 심바스타틴을 각각 고혈압치료제인 딜티아젬과 병용투여 하였을 때 경구투여된 딜티아젬의 생체이용률이 유의성 있게 증가된 것은 로바스타틴 및 심바스타틴에 의해서 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 간장에 존재하는 CYP3A 억제에 의한 딜티아젬의 대사 감소에 기인한 것으로 사료된다.

Part I. Pharmacokinetic Interaction between Lovastatin and Diltiazem in Rats

Abstract

HMG-CoA reductase inhibitors, statins and diltiazem, calcium channel blocker, could be prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. The purpose of this study was to investigate the possible effects of lovastatin, an HMG-CoA reductase inhibitor, on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats

The pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined after oral administration of diltiazem (12 mg/kg) to rats in the presence and absence of lovastatin (0.3 and 1.0 mg/kg).

Compared to the control (diltiazem alone), lovastatin significantly altered the pharmacokinetic parameters of diltiazem. The area under the plasma concentration-time curve (AUC) and the peak concentration (C_{max}) of diltiazem were significantly increased ($P < 0.05$, 1.0 mg/kg) in the presence of lovastatin. Consequently, the absolute bioavailability of diltiazem in the presence of lovastatin (10.9% at 1.0 mg/kg) were significantly higher than that of the control group ($P < 0.05$) (7.4%).

Lovastatin (1.0 mg/kg) significantly increased the AUC of desacetyldiltiazem ($P < 0.05$). Moreover, the metabolite-parent AUC ratio (MR) in the presence of lovastatin (1.0 mg/kg) was significantly decreased compared to that of the control group ($P < 0.05$). This results implied that lovastatin inhibited the metabolism of

diltiazem effectively.

Lovastatin significantly reduced rhodamine 123 efflux via P-gp in MCF-7/ADR cell overexpressing p-gp.

In conclusion, the enhanced oral bioavailability of diltiazem by lovastatin may result from decreased P-gp-mediated efflux in small intestine and inhibition of CYP 3A subfamily metabolism in small intestine or in the liver. Based on these results, if these results would be confirmed in the patients with cardiovascular diseases, the dosage of diltiazem should be readjusted when diltiazem is used concomitantly with lovastatin.

Key words: Calcium channel blocker-Diltiazem; Desacetyldiltiazem; Statin-Lovastatin; Pharmacokinetic; Bioavailability; Rats

Introduction

Diltiazem is a calcium channel blocker that is widely used in the treatment of angina, supraventricular arrhythmias and hypertension [1–3]. Diltiazem undergoes complex and extensive phase I metabolism that includes desacetylation, N-demethylation, and O-demethylation. The absolute bioavailability of diltiazem is approximately 40%, with large inter-subject variability [3,4]. In preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one-half to equivalent compared to that of diltiazem, whereas the potencies of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem were about one-third the potency of that of diltiazem [5,6]. Considering the potential contribution of active metabolites to the therapeutic outcome of diltiazem treatment, it may be important to monitor the levels of active metabolites as well as that of the parent drug in pharmacokinetic studies of diltiazem.

Cytochrome P450 (CYP) 3A, a key enzyme in the metabolism of diltiazem, is mainly localized in the liver but is also expressed in the small intestine [7–9]. Thus, diltiazem could be metabolized in the small intestine and in the liver [10–12]. Lee et al. [13] reported that the extraction ratios of diltiazem in the small intestine and liver after oral administration to rats were about 85% and 63%, respectively. This suggested that diltiazem was highly extracted in the small intestine and in the liver (first-pass metabolism). In addition to the extensive metabolism, P-glycoprotein (P-gp) may also account for the low bioavailability of diltiazem. Yusa et al. [14]

reported that calcium channel blockers such as verapamil and diltiazem competitively restrained the multi-drug resistance of P-gp. Wacher et al. [15–19] also suggested that diltiazem could act as a substrate of both CYP 3A4 and P-gp. Since P-gp is co-localized with CYP 3A4 in the small intestine, P-gp and CYP 3A4 may act synergistically to promote first-pass metabolism, resulting in the limited absorption of drugs.

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 lipoprotein (LDL) levels in patients with hypercholesterolemia [20,21]. Lovastatin is mainly metabolized by CYP 3A4 to a number of active metabolites [22,23]. Cytochrome P-450 oxidation is the primary route of phase I metabolism for lovastatin in humans and dogs [24].

Wang et al [25] 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 evaluate P-gp activity using rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells, and investigated the relationship between diltiazem, P-gp substrates and lovastatin.

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

[26,27]. But there are few reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [28,29]. Moreover, lovastatin and diltiazem could be prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. Because lovastatin and diltiazem share the same pathways in their CYP 3A-mediated metabolism, metabolism of diltiazem could be competitively inhibited by lovastatin.

Thus, the purpose of this study was to investigate the possible effects of lovastatin on the bioavailability or pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, after oral administration of diltiazem with lovastatin in rats.

Materials and Methods

Materials

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and lovastatin were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Acetonitrile, methanol and tert-butylmethylether were products from Merck Co. (Darmstadt, Germany). Other chemicals were of reagent or HPLC grade.

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 [i.v.] 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.

Intravenous and Oral Administration of Diltiazem

The rats were divided into four groups (n = 6, each): oral groups (12 mg/kg of diltiazem dissolved in water [3.0 mL/kg]) without (control) or with 0.3 and 1.0 mg/kg of lovastatin (mixed in distilled water; total oral volume of 3.0 mL/kg), and an intravenous group (4 mg/kg of diltiazem; the same solution used: 0.9% NaCl-injectable solution; total injection volume of 1.5 mL/kg). A gastric gavage tube was used to administer diltiazem and lovastatin intragastrically. Lovastatin was administered 30 min prior to oral administration of diltiazem. A blood sample (0.45-mL aliquot) was collected into a heparinized tube via the femoral artery at 0 (control), 0.016 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h for the i.v. study, and at 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h for the oral study. Whole blood (approximately 1 mL) collected from untreated rats was infused via the femoral artery at 0.25, 1, 3, and 8 h, respectively, to replace blood lost due to blood sampling. The blood samples were centrifuged (13,000 rpm, 5 min), and a 200-μL aliquots of plasma were stored at −40°C until the HPLC analysis.

HPLC assay

The plasma concentrations of diltiazem were determined using an HPLC assay by a modified from the method reported by Goebel and Kolle [30]. Briefly, 50 μL of imipramine (2 μg/mL), as the internal standard, and 1.2 mL of tert-butylmethylether were added to 0.2 mL of plasma sample. It was then mixed for 2

min using a vortex mixer and centrifuged at 13,000 rpm for 10 min. The organic layer (1 mL) was transferred to another test tube, where 0.2 mL of 0.01 N hydrochloride was added and mixed for 2 min. The water layer (50 μL) was injected into an HPLC system. The detector wavelength was set to 237 nm and the column, a μ-bondapack C18 (3.9 × 300 mm, 10 μm; Waters Co., Milford, MA, USA), was used at room temperature. A mixture of methanol:acetonitrile:0.04 M ammonium bromide:triethylamine (24:31:45:0.1, v/v/v/v, pH 7.4, adjusted with acetic acid) was used as the mobile phase at a flow rate of 1.5 mL/min. The retention times were: internal standard at 11.1 min, diltiazem at 9.6 min, and desacetyldiltiazem at 7.6 min (Figure 1). The detection limit of diltiazem and desacetyldiltiazem in rat plasma was 5 ng/mL. The coefficients of variation for diltiazem (Figure 2) and desacetyldiltiazem (Figure 3) were below 5.0%.

Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The parameter values were obtained by fitting to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The peak concentration (C_{max}) of diltiazem in plasma and the time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability

(RB) of diltiazem was calculated by $AUC_{diltiazem\ with\ lovastatin}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{desacetyldilitiazem}/AUC_{diltiazem}$.

Rhodamine-123 retention assay

The P-gp-overexpressing 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 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 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 means are presented with their standard deviation. The pharmacokinetic parameters were compared by one-way ANOVA, followed by a posteriori testing

with the use of the Dunnett correction. A P value < 0.05 was considered statistically significant.

Results

The mean plasma concentration–time profiles of diltiazem in the presence and absence of lovastatin (0.3 and 1.0 mg/kg) are shown in Figure 4. The pharmacokinetic parameters of diltiazem are summarized in Table 3. Lovastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the area under the plasma concentration–time curve from time zero to time infinity (AUC) of diltiazem by 47.2%, and the peak concentration (C_{max}) of diltiazem by 42.4%. Accordingly, the presence of lovastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the absolute bioavailability (AB%) of diltiazem in rats. Lovastatin increased the relative bioavailability (RB%) of diltiazem by 1.20- to 1.47-fold, but did not change the terminal half-life ($t_{1/2}$) and the time to reach peak concentration (T_{max}).

The plasma concentration–time profiles of desacetyldiltiazem are shown in Figure 5. The pharmacokinetic parameters of desacetyldiltiazem are summarized in Table 4. Lovastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the AUC of desacetyldiltiazem by 31.4%. However, the MR ratios were significantly ($P < 0.05$) decreased by lovastatin, suggesting that the formation of deacetyldiltiazem was considerably altered by lovastatin. Thus, the increased bioavailability of diltiazem might have been due to the inhibition of CYP 3A subfamily metabolism in the liver by lovastatin. In this study, the cell-based P-gp activity test with rhodamine-123 also showed that lovastatin (10 μM) significantly inhibited P-gp activity (Figure 6).

The enhanced oral bioavailability of diltiazem by lovastatin may result from decreased P-gp-mediated efflux in small intestine and inhibition of CYP 3A

subfamily metabolism in small intestine or in the liver

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 [31,32]. 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 [33].

Considering that the drugs used in combination therapy often share the same metabolic pathways or cellular transport pathways, there exists a 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 [26,27]. Similarly, as dual substrates of CYP 3A and P-gp, diltiazem and lovastatin may undergo the same metabolic pathways and/or cellular transport pathways after co-administration. Therefore, lovastatin could affect the bioavailability or pharmacokinetics of diltiazem in rats.

As shown in Table 3, lovastatin significantly enhanced the AUC and C_{max} of diltiazem in rats. Subsequently, the relative bioavailability (R.B.) of diltiazem was increased by 120 to 147% in the presence of lovastatin (0.3 and 1.0 mg/kg). These results were consistent with reports by Zhang et al. [34] showing that diltiazem is a

substrate of P-gp as well as CYP 3A, and that lovastatin is an effective inhibitor of P-gp and CYP 3A transport. These results were similar to the report that oral atorvastatin and fluvastatin significantly increased the bioavailability of diltiazem by inhibition of CYP 3A and P-gp in rats [28,29]. This suggested that the extraction ratio of diltiazem (first-pass metabolism) across the rat intestinal tissue was significantly reduced by P-gp and/or CYP 3A.

The AUC and C_{max} of desacetyldiltiazem were also significantly increased by 1.0 mg/kg of oral lovastatin ($P < 0.05$). However, the metabolite-parent ratio (MR) in the presence of lovastatin (1.0 mg/kg) was significantly decreased compared to that of the control group. Those results were similar to reports that oral administration of atorvastatin or fluvastatin significantyly increased the oral bioavailability o f diltiazem in rats [28,29]. Taken together, the pharmacokinetic parameters of diltiazem were significantly altered by the presence of lovastatin in rats.

Since the present study raised awareness of potential drug interactions by concomitant use of lovastatin with diltiazem, this finding has to be further evaluated in clinical studies. In the present study, cell-based P-gp activity tests with rhodamine-123 showed that lovastatin (10 μM) significantly inhibited P-gp activity (Fig. 5). This result is consistent with a report by Wang et al. [25] showing that lovastatin effectively inhibited the activity of P-gp.

Lee et al. [13] reported that the extraction ratios of diltiazem in the small intestine and liver after oral administration to rats were about 85% and 63%,

respectively. This suggested that diltiazem was highly extracted in the small intestine and in the liver, which is the same as first-pass metabolism. The increased oral bioavailability of diltiazem in the presence of lovastatin might be due to inhibition of the P-gp-mediated efflux in small intestine, and CYP 3A subfamily-mediated metabolism of diltiazem mainly in small intestine and in the liver by lovastatin.

Conclusion

The presence of lovastatin enhanced the oral bioavailability of diltiazem. Therefore, concomitant use of diltiazem with lovastatin may require close monitoring for potential drug interactions.

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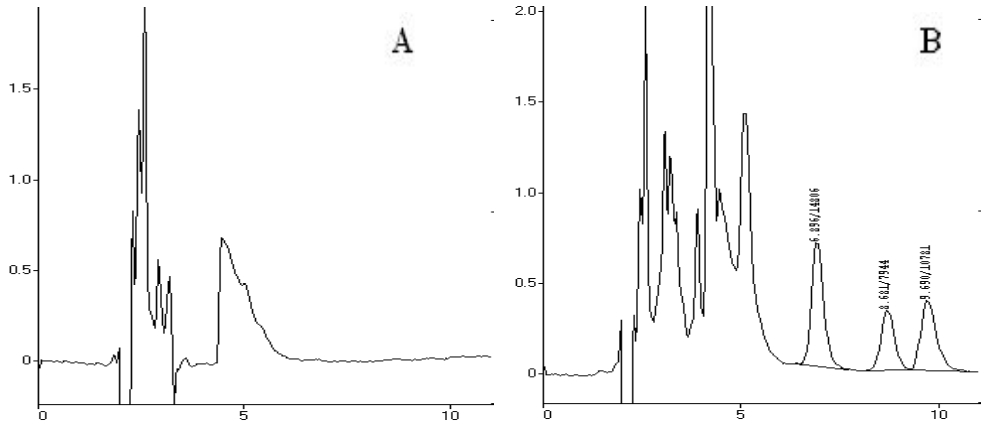

Figure 1. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with diltiazem (8.7 min), desacetyldiltiazem (6.9 min), and imipramine (internal standard; 9.7 min) (B).

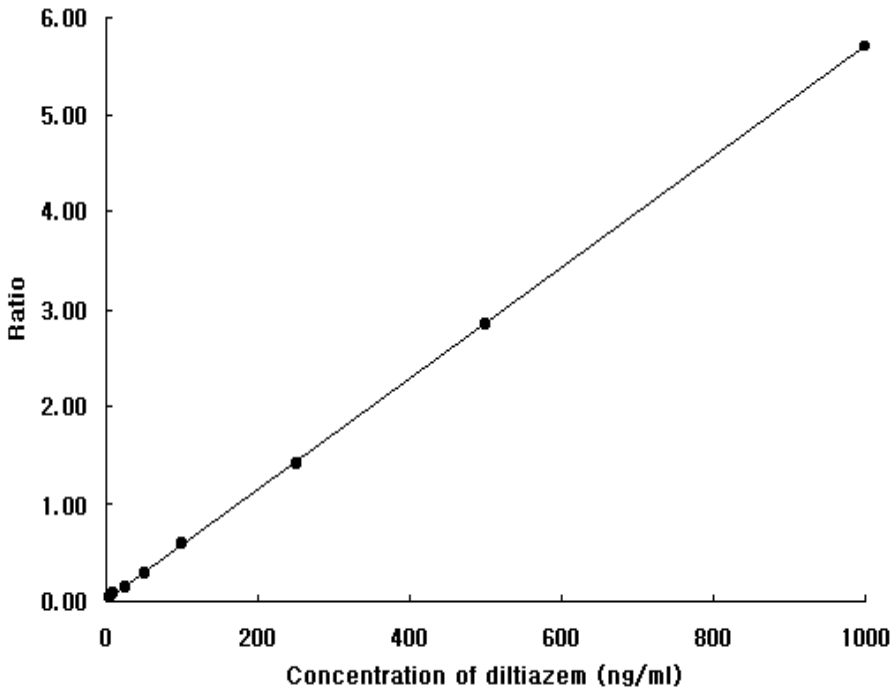

Figure 2. A calibration curve of diltiazem when spiked into the rat's blank plasma.

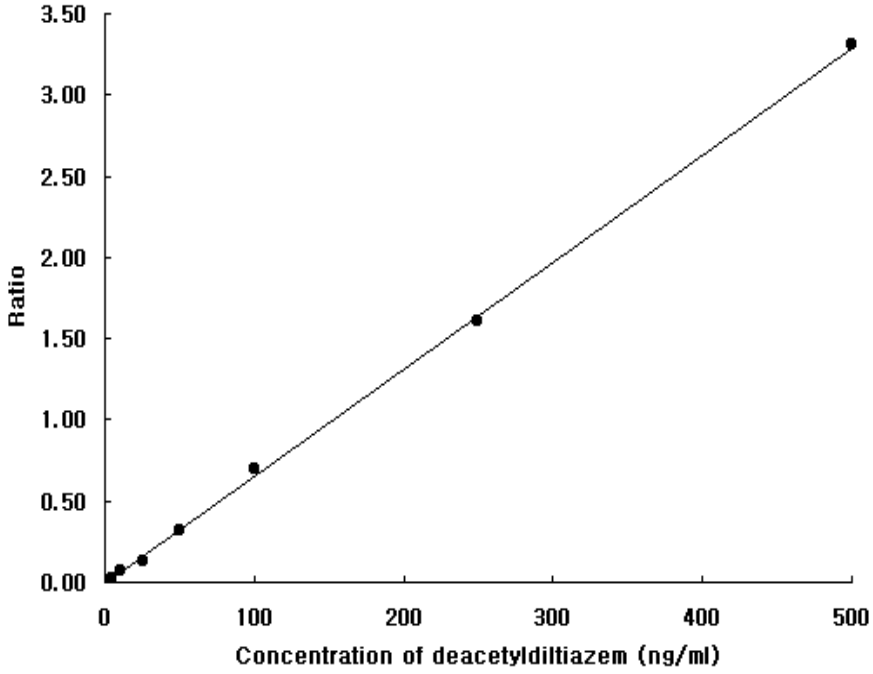

Figure 3. A calibration curve of desacetyldiltiazem when spiked into the rat's blank plasma.

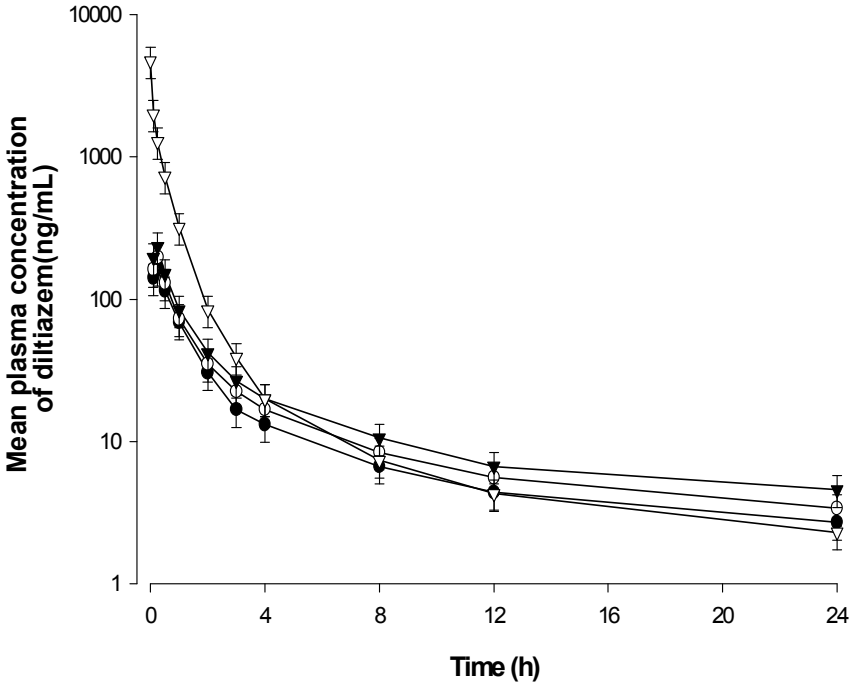

Figure 4. Mean plasma concentration-time profiles of diltiazem after intravenous (4 mg/kg) or oral (12 mg/kg) administration of diltiazem to rats in the presence and absence of lovastatin (mean ± SD, n = 6). ●: Control (diltiazem 12 mg/kg, oral); ○: presence of 0.3 mg/kg lovastatin; ▼: presence of 1.0 mg/kg lovastatin; ▽: i.v. injection of diltiazem (4 mg/kg).

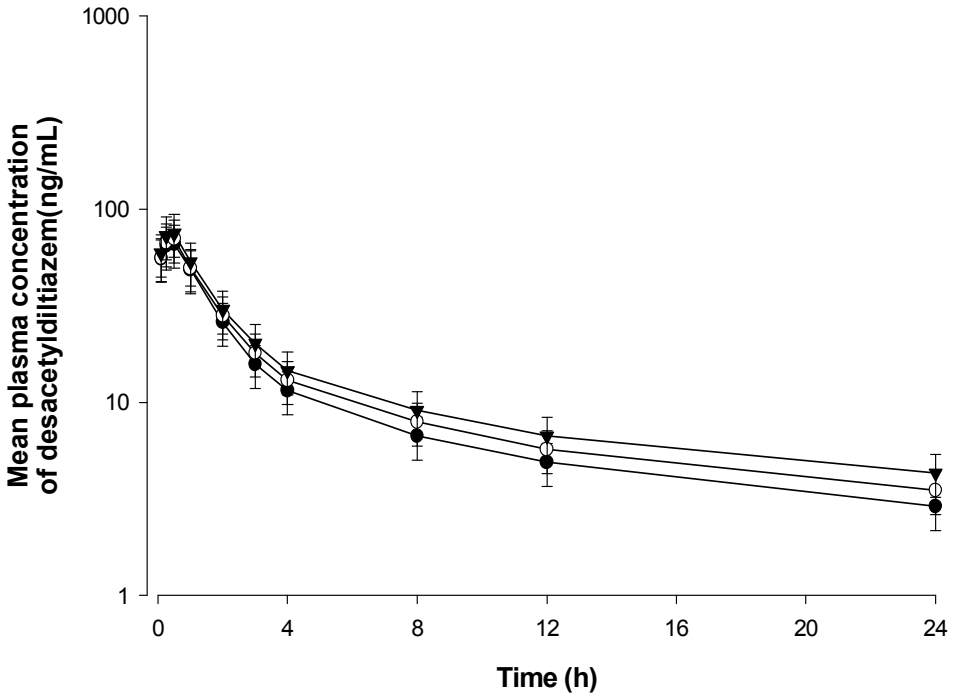

Figure 5. Mean plasma concentration-time profiles of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) to rats in the presence and absence of lovastatin (mean ± SD, *n* = 6). ●: Control (diltiazem 12 mg/kg, oral); ○: presence of 0.3 mg/kg lovastatin; ▼: presence of 1.0 mg/kg lovastatin.

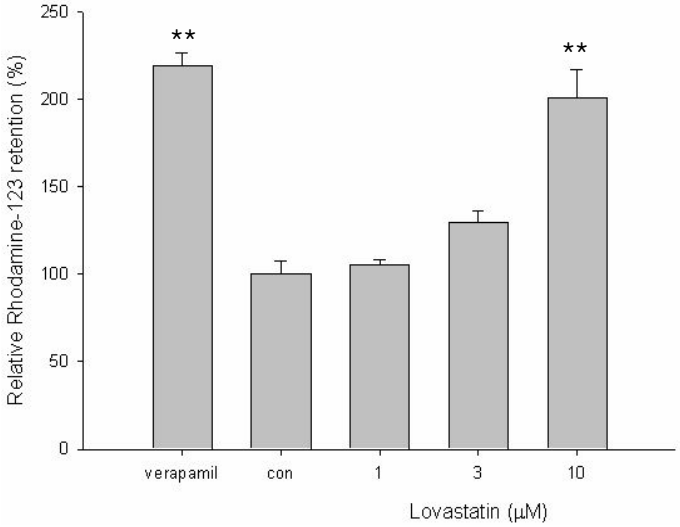

Figure 6. Rhodamine-123 (R-123) retention in lovastatin-treated MCF-7/ADR cells. After incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by the respective total protein content 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 plasma concentrations-time profiles of diltiazem after intravenous (4 mg/kg) and oral administration of diltiazem (12 mg/kg) with or without lovastatin to rats (mean ± SD, n = 6)

Time (h)	Control	Diltiazem with Lovastatin 0.3 mg/kg	Diltiazem with Lovastatin 1.0 mg/kg	i.v. (4mg/kg)
0	0	0	0	4709.0 ± 1127.3
0.1	141.0 ± 35.3	162.0 ± 40.5	196.0 ± 49.0	2003.0 ± 500.8
0.25	165.0 ± 41.3	197.0 ± 49.3	235.0 ± 58.8	1280.0 ± 320.0
0.5	115.0 ± 28.8	130.0 ± 32.6	151.0 ± 37.8	731.0 ± 182.8
1	69.0 ± 17.3	73.0 ± 18.3	84.0 ± 21.2	320.0 ± 80.0
2	30.5 ± 7.6	35.0 ± 8.8	42.0 ± 10.5	84.0 ± 21.0
3	16.8 ± 4.2	22.5 ± 5.6	26.8 ± 6.7	39.0 ± 9.8
4	13.2 ± 3.3	16.8 ± 4.3	20.0 ± 5.1	20.0 ± 5.2
8	6.7 ± 1.7	8.4 ± 2.0	10.6 ± 2.7	7.4 ± 1.9
12	4.4 ± 1.1	5.6 ± 1.5	6.7 ± 1.7	4.3 ± 1.1
24	2.7 ± 0.8	3.4 ± 0.9	4.6 ± 1.2	2.3 ± 0.6

Table 3. Mean plasma concentrations-time profiles of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) with or without of lovastatin in rats (mean ± SD, n = 6)

Time (h)	Control			Diltiazem with lovastatin					
				0.3 mg/kg			1.0 mg/kg		
0	0			0			0		
0.1	55.6	±	13.9	56.1	±	14.0	59.2	±	14.8
0.25	64.4	±	16.1	67.2	±	16.8	72.8	±	18.2
0.5	66.1	±	16.3	70.3	±	17.6	75.0	±	18.8
1	48.7	±	12.2	49.6	±	12.4	53.3	±	13.3
2	26.0	±	6.5	28.1	±	7.0	30.2	±	7.6
3	15.7	±	3.9	18.0	±	4.5	20.2	±	5.1
4	11.5	±	2.9	13.0	±	3.3	14.6	±	3.7
8	6.7	±	1.7	7.9	±	2.0	9.1	±	2.3
12	4.9	±	1.2	5.7	±	1.4	6.7	±	1.7
24	2.9	±	0.7	3.5	±	0.9	4.3	±	1.1

Table 3. Mean pharmacokinetic parameters of diltiazem after oral (12 mg/kg) administration of diltiazem with and without lovastatin in rats (mean ± SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + Lovastatin		Diltiazem (i.v.)
		0.3 mg/kg	1.0 mg/kg	
AUC (ng ·h/mL)	341.0 ± 85.3	407.9 ± 102.0	501.8 ± 125.4*	1530.2 ± 382.5
C_{max} (ng/mL)	165.0 ± 34.8	197.0 ± 44.6	235.0 ± 52.6*	
$t_{1/2}$ (h)	9.5 ±2.3	9.6± 2.4	10.3 ± 2.6	7.1 ± 1.8
T_{max} (h)	0.33 ± 0.13	0.29 ± 0.10	0.29 ± 0.10	
A.B. (%)	7.4 ±1.9	8.9 ± 2.2	10.9 ± 2.7*	
R.B. (%)	100	120	147	

*P < 0.05, significant difference compared to the control (given diltiazem alone orally). T_{max}: time to peak concentration; C_{max}: peak plasma concentration; AUC: area under the plasma concentration-time curve; $t_{1/2}$: terminal half-life; A.B.(%): absolute bioavailability; R.B.(%): relative bioavailability.

Table 4. Mean pharmacokinetic parameters of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) with and without lovastatin in rats (mean ± SD, $n = 6$)

Parameters	Diltiazem (Control)	Diltiazem with lovastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng ·h/mL)	280.2 ± 56.0	317.8 ± 63.6	368.1 ± 73.6*
C_{max} (ng/mL)	66.1 ± 13.2	70.3 ± 14.1	75.0 ± 15.0
T_{max} (h)	0.46 ±0.10	0.46 ±0.10	0.58 ±0.20
$t_{1/2}$ (h)	10.8 ± 2.2	11.3 ± 2.3	12.2 ± 2.4
M.R.	0.82 ±0.16	0.78 ± 0.16	0.73± 0.15*

*$P < 0.05$, significant difference compared to the control (oral diltiazem alone)

T_{max}: time to peak concentration; C_{max}: peak plasma concentration; AUC: area under the plasma concentration-time curve; $t_{1/2}$: terminal half-life; M.R.: Metabolite-parent AUC Ratio.

Part II. Pharmacokinetic Interaction between Simvastatin and Diltiazem in Rats

Abstract

HMG-CoA reductase inhibitors, statins and diltiazem, calcium channel blocker, could be prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. The purpose of this study was to investigate the possible effects of simvastatin, an HMG-CoA reductase inhibitor, on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats.

The pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined after oral administration of diltiazem (12 mg/kg) to rats with and without simvastatin (0.3 and 1.0 mg/kg).

Compared to the control (diltiazem alone), simvastatin significantly altered the pharmacokinetic parameters of diltiazem. The area under the plasma concentration-time curve (AUC) and the peak concentration (C_{max}) of diltiazem were significantly increased ($P < 0.05$, 0.3 and 1.0 mg/kg) with simvastatin. Consequently, the absolute bioavailability of diltiazem with simvastatin (9.9% at 0.3 mg/kg, 11.8% at 1.0 mg/kg) were significantly higher than that of the control group ($P < 0.05$) (7.4%). Simvastatin (1.0 mg/kg) significantly increased the AUC of desacetyldiltiazem ($P < 0.05$). Moreover, the metabolite-parent AUC ratio (MR) with simvastatin (1.0 mg/kg) was significantly decreased compared to that of the control group ($P < 0.05$). This results implied that simvastatin inhibited the

metabolism of diltiazem effectively. Simvastatin significantly reduced rhodamine 123 efflux via P-gp in MCF-7/ADR cell.

In conclusion, the enhanced oral bioavailability of diltiazem by simvastatin may result from decreased P-gp-mediated efflux in small intestine and inhibition of CYP 3A subfamily metabolism in small intestine or in the liver. Based on these results, if these results would be confirmed in the patients with cardiovascular diseases, the dosage of diltiazem should be readjusted when diltiazem is used concomitantly with simvastatin.

Key words: Calcium channel blocker-Diltiazem; Desacetyldiltiazem; Statin-Simvastatin; Pharmacokinetic; Bioavailability; Rats

Introduction

Diltiazem is a calcium channel blocker that is widely used in the treatment of angina, supraventricular arrhythmias and hypertension [1–3]. Diltiazem undergoes complex and extensive phase I metabolism that includes desacetylation, N-demethylation, and O-demethylation. The absolute bioavailability of diltiazem is approximately 40%, with large inter-subject variability [3,4]. In preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one-half to equivalent compared to that of diltiazem, whereas the potencies of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem were about one-third the potency of that of diltiazem [5,6]. Considering the potential contribution of active metabolites to the therapeutic outcome of diltiazem treatment, it may be important to monitor the levels of active metabolites as well as that of the parent drug in pharmacokinetic studies of diltiazem.

Cytochrome P450 (CYP) 3A, a key enzyme in the metabolism of diltiazem, is mainly localized in the liver but is also expressed in the small intestine [7–9]. Thus, diltiazem could be metabolized in the small intestine and in the liver [10–12]. Lee et al. [13] reported that the extraction ratios of diltiazem in the small intestine and liver after oral administration to rats were about 85% and 63%, respectively. This suggested that diltiazem was highly extracted in the small intestine and in the liver (first-pass metabolism). In addition to the extensive metabolism, P-glycoprotein (P-gp) may also account for the low bioavailability of diltiazem. Yusa et al. [14]

reported that calcium channel blockers such as verapamil and diltiazem competitively restrained the multi-drug resistance of P-gp. Wacher et al. [15–19] also suggested that diltiazem could act as a substrate of both CYP 3A4 and P-gp. Since P-gp is co-localized with CYP 3A4 in the small intestine, P-gp and CYP 3A4 may act synergistically to promote first-pass metabolism, resulting in the limited absorption of drugs.

Simvastatin, a HMG-CoA reductase inhibitor, is widely to treat hypercholesterolemia by lowering plasma low density lipoprotein (LDL) levels. Simvastatin is rapidly absorbed from the gastrointnstional tract after oral administration but undergoes extensive first-pass metabolism in the liver [20]. Simvastatin, administered as lactone, is metabolically activated to the open chain nonlactone simvastatin acid. This reversible conversion to the active form occurs by nonspecific carboxyesterases in the intestinal wall, liver and to some extent plasma or by nonenzymatic hydrolysis [21]. The oxidative biotransformation of simvastatin is mediated primarily by CYP3A4 [22,23], and the Center for Drug Evaluation and Research of the Food and Drug Administration has recently recommended use of simvastatin as a probe drug when studying the potential of different drugs to inhibit or induce CYP3A4 *in vivo* in human beings [24]. Simvastatin is also an inhibitor of P-gp [25,26]. Therefore, it is possible that the efficacy of diltiazem would be increased when coadministered with simvastatin. Oral diltiazem is mainly subject to CYP3A4-mediated metabolism and is a substrate for P-gp efflux. Therefore orally administered simvastatin would affect

the pharmacokinetics and metabolism of diltiazem because it is a co-substrate of CYP3A4 and an inhibitor of P-gp. Thus we attempted to evaluate P-gp activity using rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells, and investigated the relationship between diltiazem, P-gp substrates and simvastatin.

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, possibly through the inhibition of CYP 3A4 and P-gp [27]. But there are few reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [28,29]. Moreover, simvastatin and diltiazem could be prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. Because simvastatin and diltiazem share the same pathways in their CYP 3A-mediated metabolism, metabolism of diltiazem could be competitively inhibited by simvastatin.

Thus, the purpose of this study was to investigate the possible effects of simvastatin on the bioavailability or pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, after oral administration of diltiazem with simvastatin in rats.

Materials and Methods

Materials

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and simvastatin were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Acetonitrile, methanol and tert-butylmethylether were products from Merck Co. (Darmstadt, Germany). Other chemicals were of reagent or HPLC grade.

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 [i.v.] 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.

Intravenous and Oral Administration of Diltiazem

The rats were divided into four groups (n = 6, each): oral groups (12 mg/kg of diltiazem dissolved in water [3.0 mL/kg]) without (control) or with 0.3 and 1.0 mg/kg of simvastatin (mixed in distilled water; total oral volume of 3.0 mL/kg), and an intravenous group (4 mg/kg of diltiazem; the same solution used: 0.9% NaCl-injectable solution; total injection volume of 1.5 mL/kg). A gastric gavage tube was used to administer diltiazem and simvastatin intragastrically. Simvastatin was administered 30 min prior to oral administration of diltiazem. A blood sample (0.45-mL aliquot) was collected into a heparinized tube via the femoral artery at 0 (control), 0.016 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h for the i.v. study, and at 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h for the oral study. Whole blood (approximately 1 mL) collected from untreated rats was infused via the femoral artery at 0.25, 1, 3, and 8 h, respectively, to replace blood lost due to blood sampling. The blood samples were centrifuged (13,000 rpm, 5 min), and a 200-μL aliquots of plasma were stored at −40°C until the HPLC analysis.

HPLC assay

The plasma concentrations of diltiazem were determined using an HPLC assay modified from the method reported by Goebel and Kolle [30]. Briefly, 50 μL of imipramine (2 μg/mL), as the internal standard, and 1.2 mL of tert-butylmethylether were added to 0.2 mL of the plasma sample. It was then mixed

for 2 min using a vortex mixer and centrifuged at 13,000 rpm for 10 min. The organic layer (1 mL) was transferred to another test tube, where 0.2 mL of 0.01 N hydrochloride was added and mixed for 2 min. The water layer (50 μL) was injected into an HPLC system. The detector wavelength was set to 237 nm and the column, a μ-bondapack C18 (3.9 × 300 mm, 10 μm; Waters Co., Milford, MA, USA), was used at room temperature. A mixture of methanol:acetonitrile:0.04 M ammonium bromide:triethylamine (24:31:45:0.1, v/v/v/v, pH 7.4, adjusted with acetic acid) was used as the mobile phase at a flow rate of 1.5 mL/min. The retention times were: internal standard at 11.1 min, diltiazem at 9.6 min, and desacetyldiltiazem at 7.6 min. The detection limit of diltiazem and desacetyldiltiazem in rat plasma was 5 ng/mL. The coefficients of variation for diltiazem and desacetyldiltiazem were below 5.0%.

Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The parameter values were obtained by fitting to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration–time curve (AUC) was calculated by a trapezoidal rule. The peak concentration (C_{max}) of diltiazem in plasma and time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability

(RB) of diltiazem were calculated by $AUC_{diltiazem\ with\ simvastatin}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{desacetyldilitiazem}/AUC_{diltiazem}$.

Rhodamine-123 retention assay

The P-gp-overexpressing 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 simvastatin (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 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 means are presented with their standard deviation. The pharmacokinetic parameters were compared by one-way ANOVA, followed by a posteriori testing

with the use of the Dunnett correction. A P value < 0.05 was considered statistically significant.

Results

The mean plasma concentration–time profiles of diltiazem in the presence and absence of simvastatin (0.3 and 1.0 mg/kg) are shown in Figure 10. The pharmacokinetic parameters of diltiazem are summarized in Table 7. Simvastatin (0.3 and 1.0 mg/kg) significantly ($P < 0.05$) increased the area under the plasma concentration–time curve from time zero to time infinity (AUC) of diltiazem by 33.3 and 58.9%, and the peak concentration (C_{max}) of diltiazem by 32.1 and 49.1% respectively. Accordingly, the presence of simvastatin (0.3 and 1.0 mg/kg) significantly ($P < 0.05$) increased the absolute bioavailability (AB%) of diltiazem in rats. Simvastatin increased the relative bioavailability (RB%) of diltiazem by 1.33- to 1.59-fold, but did not change the terminal half-life ($t_{1/2}$) and the time to reach peak concentration (T_{max}).

The plasma concentration–time profiles of desacetyldiltiazem are shown in Figure 11. The pharmacokinetic parameters of desacetyldiltiazem are summarized in Table 8. The presence of simvastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the AUC of desacetyldiltiazem by 37.4%. However, the MR ratios were significantly ($P < 0.05$, 1.0 mg/kg) decreased by simvastatin, suggesting that the formation of desacetyldiltiazem was considerably altered by simvastatin. Thus, the increased bioavailability of diltiazem by simvastatin might have been due to the inhibition of CYP3A subfamily in the liver. In this study, the cell-based P-gp activity test with rhodamine-123 also showed that simvastatin (10 μM) significantly inhibited P-gp activity (Figure 12). The enhanced oral bioavailability

of diltiazem by simvastatin may result from decreased P-gp-mediated efflux in small intestine and inhibition of CYP 3A subfamily metabolism in small intestine or in the liver

Discussion

The enterocytes contain virtually all types of drug metabolizing enzymes that are found in the liver. The importance of hepatic metabolism for limiting systemic drug availability is well established; however, intestinal drug metabolism can further diminish systemic availability. Through functional enzyme activity studies and immunoblot analysis, CYP 3A expression in mature enterocytes, located mainly in the villi tips, of jejunal mucosa was shown to be comparable to or may even exceed the expression of CYP 3A in hepatocytes [31]. Total CYP P450 content increased slightly proceeding from the duodenum to the jejunum and then decreased sharply towards to the ileum [32]. Using in situ hybridization with a probe specific for CYP 3A4, McKinnon confirmed CYP 3A expression throughout the entire small intestine, with highest levels in its proximal regions [33]. The most abundant CYP isoenzyme in the intestine is 3A4 and 3A5 [21].

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, CYP 3A4 and P-gp have been recognized as a concerted barrier to drug absorption [34,35]. 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 [36].

Considering that the drugs used in combination therapy often share the same metabolic pathways or cellular transport pathways, there exists a 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 simvastatin [27]. Similarly, as dual substrates of CYP 3A and P-gp, diltiazem and simvastatin may undergo the same metabolic pathways and/or cellular transport pathways after co-administration. Therefore, simvastatin could affect the bioavailability or pharmacokinetics of diltiazem in rats.

As shown in Table 7, simvastatin significantly enhanced the AUC and C_{max} of diltiazem in rats. Subsequently, the relative bioavailability (R.B.) of diltiazem was increased by 133 to 159% in the presence of simvastatin (0.3 and 1.0 mg/kg). These results were consistent with the report that oral atorvastatin and fluvastatin significantly increased the bioavailability of diltiazem by inhibition of CYP 3A and P-gp in rats [28,29]. This suggested that the extraction ratio of diltiazem across the rat intestinal tissue was significantly reduced by P-gp and/or CYP 3A. These results were similar to reports by Marumo et al. [37] showing that simvastatin enhanced the hypotensive effect of diltiazem in rats.

The AUC and C_{max} of desacetyldiltiazem were also significantly increased by 1.0 mg/kg of oral simvastatin ($P < 0.05$). However, the metabolite-parent ratio (MR) in the presence of simvastatin was significantly ($P < 0.05$, 1.0 mg/kg) decreased compared to that of the control group. Those results were similar to reports that ora l administration of atorvastatin or fluvastatin significantyly increased the oral bioavailability of diltiazem in rats [28,29]. Taken together, the pharmacokinetic

parameters of diltiazem were significantly altered by the presence of simvastatin in rats. In the present study, cell-based P-gp activity tests using rhodamine-123 showed that simvastatin (10 μM) significantly inhibited P-gp activity (Figure 12). This result is consistent with a report by Bogman et al. [26] showing that simvastatin effectively inhibited the activity of P-gp.

Lee et al. [13] reported that the extraction ratios of diltiazem in the small intestine and liver after oral administration to rats were about 85% and 63%, respectively. This suggested that diltiazem was highly extracted in the small intestine and in the liver, which is the same as first-pass metabolism. The increased oral bioavailability of diltiazem in the presence of simvastatin might be due to inhibition of the P-gp-mediated efflux in small intestine, and CYP 3A subfamily-mediated metabolism in small intestine and in the liver by simvastatin.

Conclusion

The presence of simvastatin enhanced the bioavailability of diltiazem. Therefore, concomitant use of diltiazem with simvastatin may require close monitoring for potential drug interactions.

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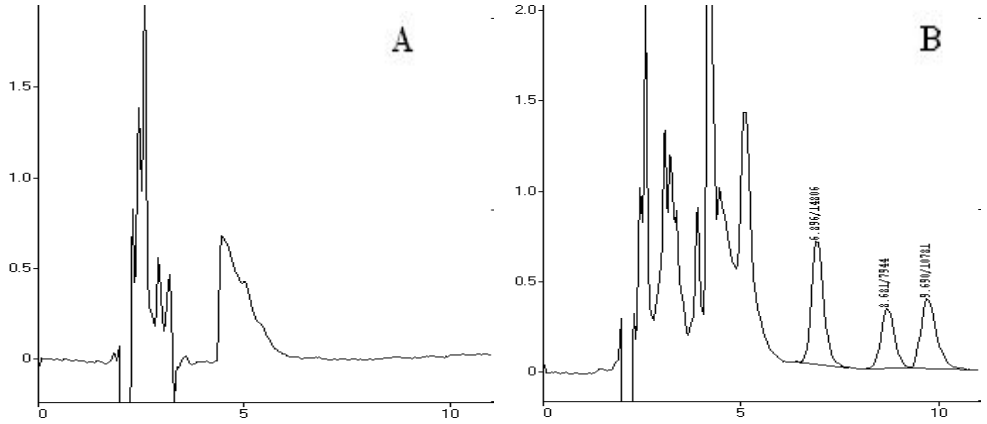

Figure 7. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with diltiazem (8.7 min), desacetyldiltiazem (6.9 min), and imipramine (internal standard; 9.7 min) (B).

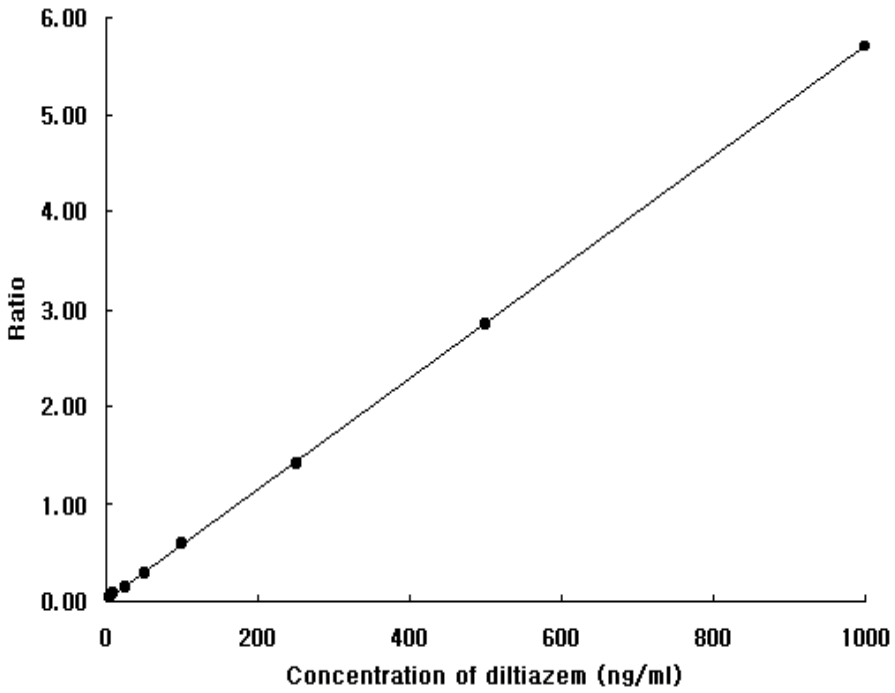

Figure 8. A calibration curve of diltiazem when spiked into the rat's blank plasma.

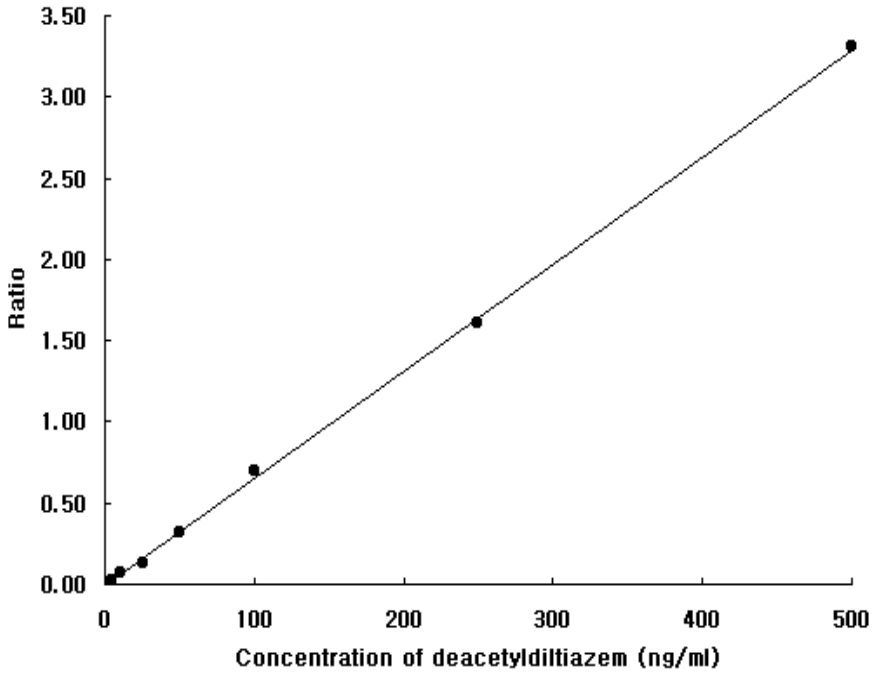

Figure 9. A calibration curve of desacetyldiltiazem when spiked into the rat's blank plasma.

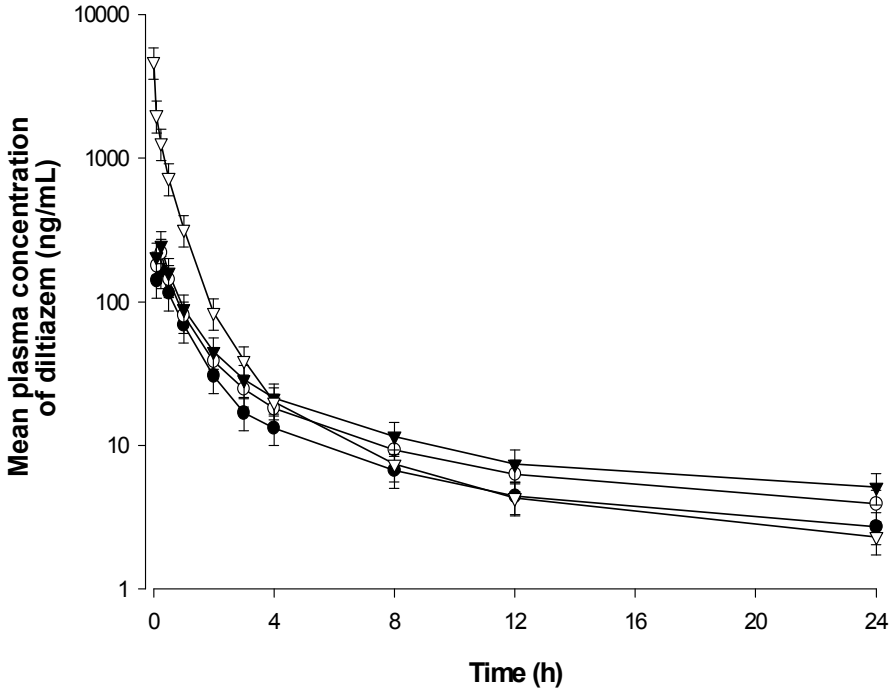

Figure 10. Mean plasma concentration-time profiles of diltiazem after intravenous (4 mg/kg) or oral (12 mg/kg) administration of diltiazem to rats in the presence and absence of simvastatin (mean ± SD, *n* = 6). ●: Control (diltiazem 12 mg/kg, oral); ○: presence of 0.3 mg/kg simvastatin; ▼: presence of 1.0 mg/kg simvastatin; ▽: i.v. injection of diltiazem (4 mg/kg).

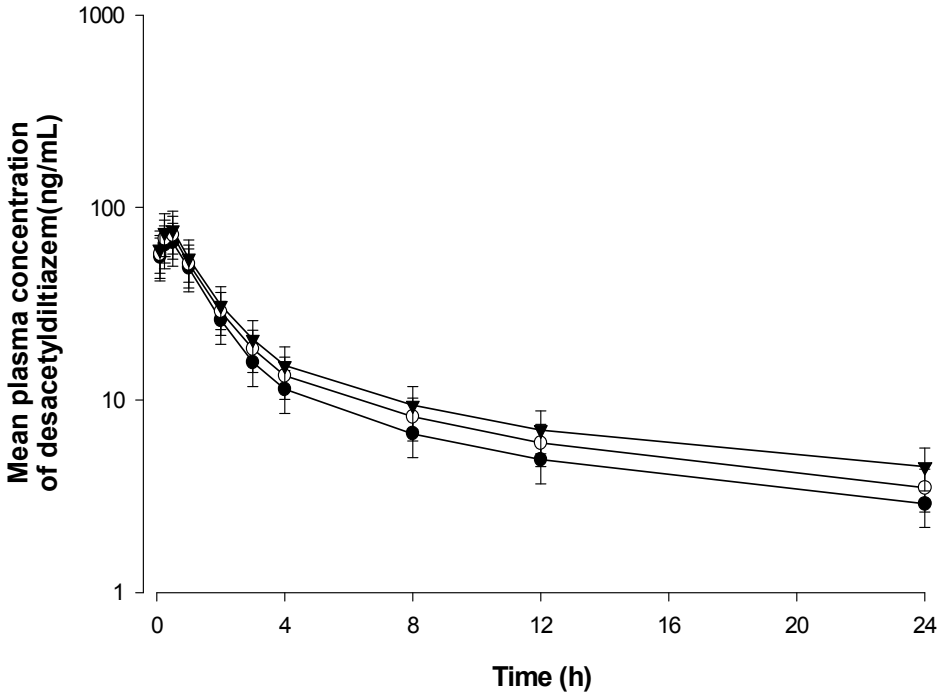

Figure 11. Mean plasma concentration-time profiles of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) to rats in the presence and absence of simvastatin (mean ± SD, n = 6). ●: Control (diltiazem 12 mg/kg, oral); ○: presence of 0.3 mg/kg simvastatin; ▼: presence of 1.0 mg/kg simvastatin.

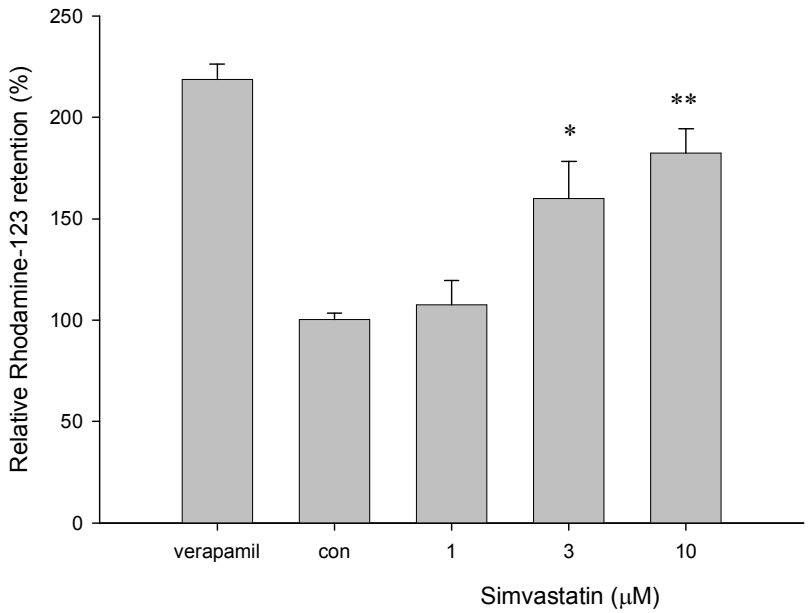

Figure 12. Rhodamine-123 (R-123) retention in simvastatin-treated MCF-7/ADR cells. After incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by the respective total protein content 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 plasma concentrations-time profiles of diltiazem after oral administration of diltiazem (12 mg/kg) with and without simvastatin to rats (mean ± S.D. $n = 6$)

Time (h)	Control	Diltiazem with Simvastatin 0.3 mg/kg	Diltiazem with Simvastatin 1.0 mg/kg	i.v. (4mg/kg)
0	0	0	0	4709 ± 1127.3
0.1	141.0 ± 35.2	178.0 ± 44.5	205.0 ± 51.2	2003.0 ± 500.8
0.25	165.0 ± 41.2	218.0 ± 54.5	246.0 ± 61.5	1280.0 ± 320.0
0.5	115.0 ± 28.8	143.0 ± 35.8	160.0 ± 40.0	731.0 ± 182.8
1	69.0 ± 17.1	80.0 ± 20.0	89.0 ± 22.2	320.0 ± 80.0
2	30.5 ± 7.6	38.5 ± 9.6	44.9 ± 11.2	84.0 ± 21.0
3	16.8 ± 4.2	24.7 ± 6.1	28.6 ± 7.1	39.0 ± 9.8
4	13.2 ± 3.3	18.1 ± 4.5	21.3 ± 5.3	20.0 ± 5.2
8	6.7 ± 1.7	9.3 ± 2.3	11.5 ± 2.8	7.4 ± 1.9
12	4.4 ± 1.1	6.3 ± 1.5	7.4 ± 1.9	4.3 ± 1.1
24	2.7 ± 0.7	3.9 ± 0.9	5.1 ± 1.2	2.3 ± 0.6

Table 6. Mean plasma concentrations-time profiles of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) with or without of simvastatin in rats (mean ± S.D. $n = 6$)

Time (h)	Control	Diltiazem with simvastatin	
		0.3 mg/kg	1.0 mg/kg
0	0	0	0
0.1	55.6 ± 13.9	57.4 ± 14.4	60.7 ± 15.2
0.25	64.4 ± 16.1	68.8 ± 17.2	74.3 ± 18.6
0.5	66.1 ± 16.5	72.1 ± 18.0	76.7 ± 19.2
1	48.7 ± 12.2	51.0 ± 12.8	54.5 ± 13.6
2	26.0 ± 6.5	28.9 ± 7.2	31.1 ± 7.8
3	15.7 ± 3.9	18.5 ± 4.6	20.7 ± 5.2
4	11.4 ± 2.8	13.4 ± 3.4	15.1 ± 3.8
8	6.7 ± 1.6	8.2 ± 2.1	9.4 ± 2.4
12	4.9 ± 1.2	6.0 ± 1.5	7.0 ± 1.8
24	2.9 ± 0.7	3.5 ± 0.9	4.5 ± 1.1

Table 7. Mean pharmacokinetic parameters of diltiazem after intravenous (4 mg/kg) and oral (12 mg/kg) administration of diltiazem with and without simvastatin in rats (mean ± SD, n = 6).

Parameters	Diltiazem (Control)	Diltiazem + Simvastatin		Diltiazem (i.v.)
		0.3 mg/kg	1.0 mg/kg	
AUC (ng ·h/mL)	341.0 ± 68.2	454.4 ± 90.9*	541.9 ± 108.4*	1530.2 ± 306.0
C_{max} (ng/mL)	165.0 ± 33.0	218.0 ± 43.6*	246.0 ± 49.2*	
$t_{1/2}$ (h)	9.5 ± 1.9	9.9 ± 2.0	10.6 ± 2.1	7.1 ± 1.4
T_{max} (h)	0.29± 0.10	0.33 ±0.13	0.33 ±0.13	
A.B. (%)	7.4 ±1.5	9.9 ± 2.0*	11.8 ± 2.4*	
R.B. (%)	100	133	159	

*P < 0.05, significant difference compared to the control (diltiazem alone).

T_{max}: time to peak concentration; C_{max}: peak plasma concentration; AUC: area under the plasma concentration-time curve; $t_{1/2}$: terminal half-life; A.B.(%): absolute bioavailability; R.B.(%): relative bioavailability.

Table 8. Mean pharmacokinetic parameters of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) with and without simvastatin in rats (mean ± SD, $n = 6$)

Parameters	Diltiazem (Control)	Diltiazem with simvastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng ·h/mL)	280.2 ± 56.0	324.3 ± 64.9	382.0 ± 76.4*
C_{max} (ng/mL)	66.1 ± 13.2	72.1 ± 14.4	76.7 ± 15.3
T_{max} (h)	0.46 ±0.10	0.46 ±0.10	0.58 ±0.20
$t_{1/2}$ (h)	10.9 ± 2.2	11.0 ± 2.2	12.3 ± 2.5
M.R.	0.82 ±0.16	0.71 ± 0.14	0.70 ± 0.14*

*$P < 0.05$, significant difference compared to control (diltiazem alone)

T_{max}: time to peak concentration; C_{max}: peak plasma concentration; AUC: area under the plasma concentration-time curve; $t_{1/2}$: terminal half-life; M.R.: Metabolite-parent AUC Ratio.

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 홍순표 교수님께 머리숙여 감사를 드립니다.

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

부족한 저의 학위논문을 지적 및 보완하여 심사하여 주신 서울대학교 손대원 교수님과 전남대학교 정명호 교수님께 감사를 드립니다.

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

항상 기도해 주시고 염려해 주신 양가 부모님께 한없는 감사를 드립니다.

그리고 항상 저의 옆에서 고생을 아끼지 않은 사랑하는 아내와 아들에게 고마움을 전하고 싶읍니다.

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

저작물 이용 허락서

학 과	의학과	학 번	20047418	과 정	박사
성 명	한 글 : 최동현	한 문 :		영 문 : Dong-Hyun Choi	
주 소	광주시 남구 봉선동 포스코 102 동 2001 호				
연락처	E-mail : dhchoi@paran.com				
논문제목	한글: 흰쥐에서 심바스타틴 및 로바스타틴과 딜티아젬과의 약물상호작용. 영문: Drug interaction between lovastatin or simvastatin and diltiazem 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: 최동현 (서명 또는 인)

조선대학교 총장 귀하