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碩士學位論文

Effect of fluvastatin on the Pharmacokinetics of diltiazem and its metabolite, desacetyldiltiazem in Rats

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藥 學 科

朴 永 吉

朴永吉의 碩士學位論文을 認准함

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Abstract

The aim of this study is to investigate the effect of fluvastatin, on the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, in rats. Pharmacokinetic parameters of diltiazem and desacetyldiltiazem in plasma were determined after an oral administration of diltiazem (15 mg/kg) to rats in the presence or absence of fluvastatin (0.5, 1.5 and 3.0 mg/kg). Compared with the control (given diltiazem alone), the presence of fluvastatin significantly increased the AUC, C_{max} and K_a of diltiazem ($p<0.05$). AB% of diltiazem was increased significantly ($p<0.05$), and RB% of diltiazem increased from 1.36-to 1.77-fold. But there were no significant change in T_{max} , K_{el} and $T_{1/2}$ of diltiazem. The presence of fluvastatin also altered the pharmacokintic parameters of desacetyldiltiazem. Coadministration of fluvastatin significantly ($p<0.05$) increased the AUC of desacetyldiltiazem, whereas the MR of desacetyldiatiazem was decreased significantly ($p<0.05$) by the fluvastatin dose of 1.5 and 3.0 mg/kg, which means fluvastatin might inhibits the metabolism of diltiazem.

The presence of fluvastatin enhanced the bioavailability of diltiazem. In the dose range of 0.5 to 3.0 mg/kg, the more increasing the dose of fluvastatin the more affected the oral diltiazem. If the results are further testified in the clinical trial, diltiazem dose should be adjusted when dilitazem is coadministered with fluvastatin for the drug interaction between them.

Key words: diltiazem, desacetyldiltiazem, pharmacokinetics, fluvastatin, rat

국 문 초 록

흰쥐에서 프루바스타틴이 딜티아젬 및 그 대사체인 데스아세칠딜티아젬의 약물동태에 미치는 영향

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고혈압 및 고지혈증 환자에서 프루바스타틴과 딜티아젬의 병용처방이 가능하다. 그러므로 프루바스타틴이 딜티아젬과 데스아세칠딜티아젬의 약물동태 파라미터에 미칠것으로 사료되어 딜티아젬 15 mg/kg 과 프루바스타틴 (0.5, 1.5, 3.0 mg/kg)을 흰쥐에 경구병용투여하여 본 연구를 실시하였다.

프루바스타틴은 딜티아젬의 AUC, C_{max}, K_a 와 생체이용률을 유의성 있게 증가시켰다. 그 결과로 상대적생체이용률을 1.36-1.77 배 증가되였다. 프루바스타틴은 딜티아젬의 대사체인 데스아세칠딜티아젬의 동태파라미터에 영향을 미쳤다. 즉 데스아세칠딜티아젬의 AUC 을 유의성 있게 증가시켰으며 MR (대사률)은 유의성 (1.5 와 3.0 mg/kg) 있게 감소시켰다. 이것은 프루바스타틴이 딜티아젬의 대사를 억제시킨 것으로 사료된다.

프루바스타틴의 용량을 증가와 더불어 딜티아젬의 생체이용률도 증가되었다. 임상에서 프루바스타틴과 딜티아젬을 병용투여시 딜티아젬의 용량를 조절하는 것이 바람직하다고 사료된다.

Introduction

Diltiazem is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension [1-3]. Diltiazem undergoes the extensive and complex phase I metabolism including desacetylation, N-demethylation, and O-demethylation and the absolute bioavailability is approximately 40%, with a large inter-subject variability [3-4]. In the preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one half of equivalent compared with diltiazem, whereas the potencies of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem were about one third the potency 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 active metabolites as well as the parent drug in the pharmacokinetic studies of diltiazem. CYP 3A4, a key enzyme for the metabolism of diltiazem is mainly located in liver, but it is also expressed in small intestine [7-9]. Thus, diltiazem could be metabolized in small intestine as well as in liver [10-12]. Lee *et al.* has reported that the extraction ratios of diltiazem in small intestine and liver after an oral administration to rats were about 85% and 63%, respectively, suggesting that diltiazem is highly extracted in the small intestine as well as in the liver [13]. Diltiazem is not only a multi-drug resistance (MDR) modulator but also a substrate for the efflux of P-gp. In vitro study using rat intestine suggested that diltiazem is a P-gp substrate [14]. In the small intestine, P-gp is co-localized at the apical membrane of the epithelial cells with CYP 3A4 [15,16]. P-gp and CYP 3A4 might act

synergistically to the presystemic drug metabolism by circulating the substrates of P-gp between the lumen and epithelial cells, leading to prolonged exposure to CYP 3A4, resulting in a reduced absorption of the drug.

Fluvastatin, the first marketed synthetic hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitor, is an antilipemic agent and also used as an adjunct to dietary therapy to slow the progression of coronary atherosclerosis in hypercholestrolemic patients with coronary heart disease. It acts as a part of treatment strategy help lowering the total and LDL-cholesterol concentrations to target level [17 18 19]. It is rapidly and completely absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver[20]. Fluvastatin, is mainly metabolized by the CYP 2C9 as well as CYP 3A4 [21, 22, 23, 24]. Hypertension agents are commonly coadministered with cholesterol-lowering agents in clinics. There are some reports about the effect of calcium channel antagonists on the pharmacokinetcs of HMG-CoA reductase inhibitors. Calcium-channel blockers increased plasma concentrations of some statins, probably by inhibition of cytochrome P450 (CYP 2C9, CYP 3A4). Pharmacokinetic studies have reported the increased plasma concentrations of simvastatin by verapamil and diltiazem[25]. A retrospective study found that the cholesterol-lowering effect of simvastatin was greater in patients who were also receiving diltiazem, and there have also been two reports of rhabdomysis, associated with hepatitis in patients receiving simvastatin with diltiazem together [26]. Statins (Atorvastatin, lovastatin, fluvastatin and simvastatin) inhibited P-glycoprotein mediated

R123 transport in a concentration-dependent manner.[27] Fluvastatin as one of the statins, substrates for both cytochrome P450 (CYP 2C9, CYP 3A4) metabolism and P-glycoprotein efflux[28], when it is administered with diltiazem, it might affect the pharmacokinetics of diltiazem. But there are no reports about the effect of HMG-CoA reductase inhibitors on the pharmacokinetcs of calcium channel antagonists.

So, this study aimed to investigate the effect of fluvastatin on the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem in rats.

Materials and Methods

A. Materials

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and fluvastatin were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, tert-butylmethylether were obtained from Merck Co. (Darmstadt, Germany). All other chemicals were reagent grade and all solvents were HPLC grade.

B. Animal Studies

Female Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and given a normal standard chow diet (No. 322-7-1) purchased from Superfeed Co. (Gangwon, Korea) and tap water *ad libitum*. Throughout the experiment, the animals were housed in laminar flow cages, three per cage, which was maintained at 22±2 ℃, 50-60% relative humidity, under a 12 h light-dark cycle. The animals were allowed to acclimatize for at least one week prior to the experiments. This experiments were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal care committee at our institution (Chosun University) approved this study.

The rats were divided into five groups of six each: the control group (15

mg/kg diltiazem, oral), three coadministration groups (15 mg/kg diltiazem orally coadministered with fluvastatin 0.5, 1.5 or 3.0 mg/kg), and an IV group (intravenous administration of 5.0 mg/kg diltiazem). The rats were fasted for at least 24 h prior to the experiments, but given free access to water. Each rat was anaesthetized with diethyl ether. The right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) to allow for blood sampling.

In the previous study reported by Yeung et al., the oral administration of diltiazem to rats at 15 mg/kg achieved the plasma level comparable to the therapeutic concentrations in humans [29]. So, 15 mg/kg of diltiazem was chosen as the control group in this study. The drug used in the control group was prepared by adding diltiazem to distilled water (2 ml/rat). The mixtures for coadministered group were prepared by mixing diltiazem (15 mg/kg) and the required fluvastatin (0.5, 1.5, 3.0 mg/kg) in distilled water (2 ml). Blood samples were collected from the femoral artery at 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 hr postdose. Blood samples were centrifuged and the plasma was removed and stored at -40℃ until analyzed by HPLC.

C. HPLC Assay

The plasma concentrations of diltiazem were determined by the HPLC assay modified from the method of Goebel et al. [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 samples. The mixture was then stirred for 2 min and centrifuged for 10 min. 1 ml of the organic layer was transferred to a clean test tube and 0.2 mL of 0.01N hydrochloride was added and mixed for 2 min. 50 μL of the water layer were injected into the HPLC system. The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The UV detector was set to 237 nm. The stationary phase was a μ-bondapack C_{18} column (3.9 × 300 mm, 10 μm, Waters Co., Ireland) and the mobile phase was methanol: acetonitrile: 0.04 M ammonium bromide: triethylamine (24: 31: 45: 0.1, v/v/v, pH 7.4, adjusted with acetic acid). The retention times at a flow rate of 1.5 mL/min are as follows: desacetyldiltiazem at 6.9-min, diltiazem at 8.7-min and internal standard at 9.7-min (Fig 1). The calibration curves of diltiazem and desacetyldiltiazem were linear within the range of 10-1000 ng/mL (Fig 2). The calibration curves of diltiazem and desacetyldiltiazem were linear within the range of 5-500 ng/mL (Fig 3). The intra-day (n=5) and inter-day (n=5) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem, and 1.5% for imipramine.

D. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed by the

LAGRAN method using the LARGAN computer program [31]. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed values from the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($T_{1/2}$) of the drug was obtained with $0.693/K_{el}$. The absolute bioavailability (A.B.%) of diltiazem was calculated by $AUC_{oral}/AUC_{iv} \times Dose_{i.v}/Dose_{oral} \times 100$, and the relative bioavailability (R.B.%) of diltiazem was estimated by $AUC_{diltiazem\ with\ fluvastatin} / AUC_{control} \times 100$. The metabolite-parent ratio (M.R.) was estimated by $AUC_{desacetyldiltiazem} /AUC_{diltiazem}$.

E. Statistical analysis

All means were presented with their standard deviation (Mean ± S.D.). Unpaired Student's t-test was utilized to determine a significant difference between the group of control and coadministrations. Differences were considered to be significant at $p < 0.05$.

Results

The mean plasma concentration-time profiles of diltiazem in the presence

and absence of fluvatatin were characterized in rats and illustrated in Table 1 and Figure 4. The mean pharmacokinetic parameters of diltiazem were also summarized in Table 3.

As shown in Table 3, the coadministration of fluvastatin (0.5, 1.5 or 3.0 mg/kg) significantly altered the pharmacokinetic parameters of diltiazem compared to the control group (given diltiazem alone). The absorption rate constant (K_a), peak concentration (C_{max}) and the areas under the plasma concentration time curve (AUC) of diltiazem were significantly ($p<0.05$) increased in the rats coadministrated with fluvastatin, especially by 3.0 mg/kg ($p<0.01$). There was no significant change in the time to peak concentration (T_{max}) and terminal plasma half-life ($t_{1/2}$) of diltiazem in the presence of fluvastatin. The absolute bioavailability values (AB%) of diltiazem in the rats coadministered with fluvastatin ranges from 8.4 to 13.2, which was significantly higher ($p<0.05$) than those from the control group (6.1). The relative bioavailability (RB%) of diltiazem increased from 1.36-to 1.77-fold.

The pharmacokinetic profiles of desacetyldiltiazem were also evaluated in the presence and absence of fluvastatin (Table 4 and Figure 5). As summarized in Table 2, the oral exposure of desacetyldiltiazem increased significantly ($p<0.05$) in the presence of fluvastatin (0.5, 1.5 and 3.0 mg/kg). However, the metabolite-parent ratio (M.R.) in the rats coadministered with fluvastatin (1.5 and 3.0 mg/kg) decreased by approximately 20% compared to the control group.

Discussion

Based on the broad overlap in the substrate specificities as well as co-localization in the small intestine, the primary site of absorption for orally

administered drugs, cytochrome P450 and P-gp have been recognized as a concerted barrier to the drug absorption [32, 33]. Therefore, dual inhibitors against both CYP 3A4 and P-gp should have a great impact on the bioavailability of many drugs where CYP 3A4 metabolism as well as P-gp mediated efflux is the major barrier to the systemic availability. Besides the extensive metabolism by CYP 3A4, diltiazem appeared to be the substrate of P-gp, suggesting that P-gp and CYP 3A4 should act synergistically to limit the oral bioavailability of diltiazem [34, 35].

In this study, coadministration of fluvastatin significantly enhanced K_a of diltiazem, and the C_{max} and AUC of diltiazem increased by 43 to 122%. AB% of diltiazem ranges from 8.4 to 13.2, and also significantly higher ($p<0.05$) than the control group (6.1). Fluvastatin is predominantly (50-80%) metabolized by CYP 2C9 and CYP 3A4 (~20%), with less contribution via CYP 2C8 (~5%) [21,36], and it inhibited the P-glycoprotein mediated R123 transport in a murine monocytic leukemia cell line system [27]. The increased oral bioavailability of might be contribute to fluvastatin, which as a substrate of cytochrome P450 (CYP 2C9, CYP 3A4) and a inhibitor of P-glycoprotein, via the competition of cytochrome P450 (CYP 2C9, CYP 3A4) mediated metabolism and inhibition of P-glycoprotein both in the intestine and liver. These results are similar to the observation from the previous study, in that fluvastatin increased blood concentration of cyclosporin, a substrate of CYP 3A4 [37].

Although coadministration of fluvastatin significantly increased the active

metabolite of diltiazem, desacetyldiltiazem, the decreased M.R. of desacetyldiltiazem by fluvastatin also support the speculation that although fluvastatin mainly substrates for CYP 2C9 metabolism and CYP 3A4.

Based on the results acquired from this study, fluvastatin would enhance the oral bioavailability of diltiazem in rats.

Conclusion

Coadministration of fluvastatin significantly enhanced the systemic bioavailability e of diltiazem in rats. If the results are further confirmed in the clinic trial, it shoud be taken into consideration when diltiazem is treated with

concomitantly with fluvastatin to the patients.

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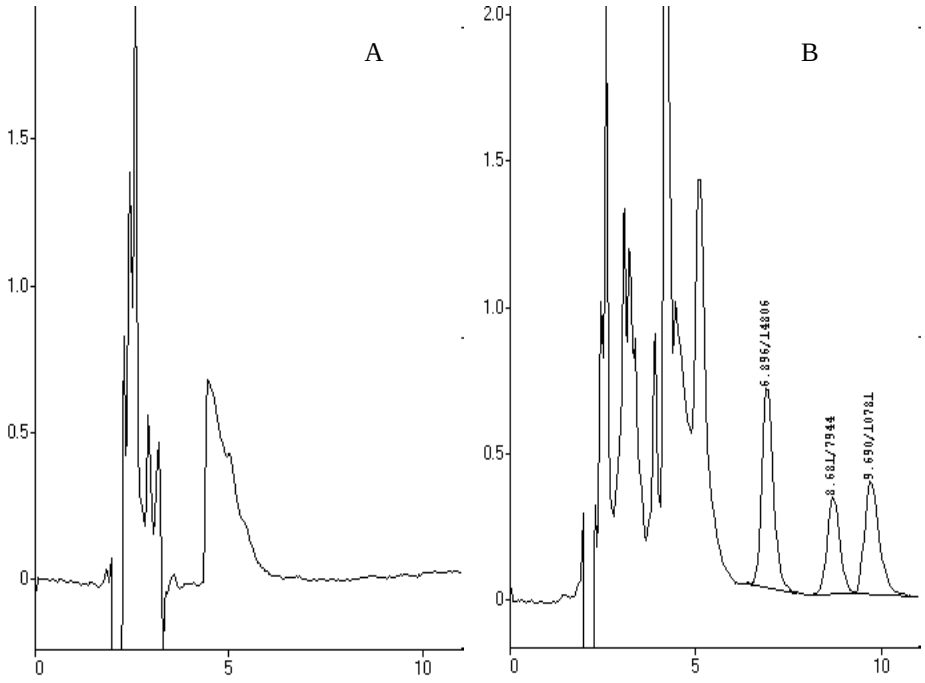

Figure 1. Chromatogram of rat's blank plasma (A) and the plasma (B) spiked with desacetyldiltiazem (6.9 min), diltiazem (8.7 min) and the internal stand, imipramine (9.7 min).

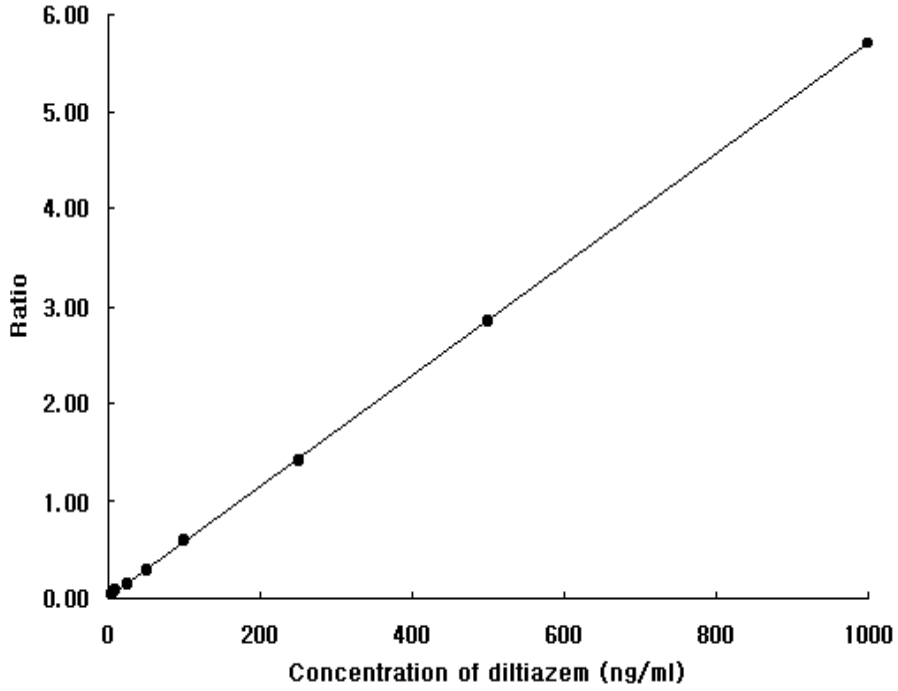

Figure 2. Calibration curve of diltiazem spiked in rat plasma.

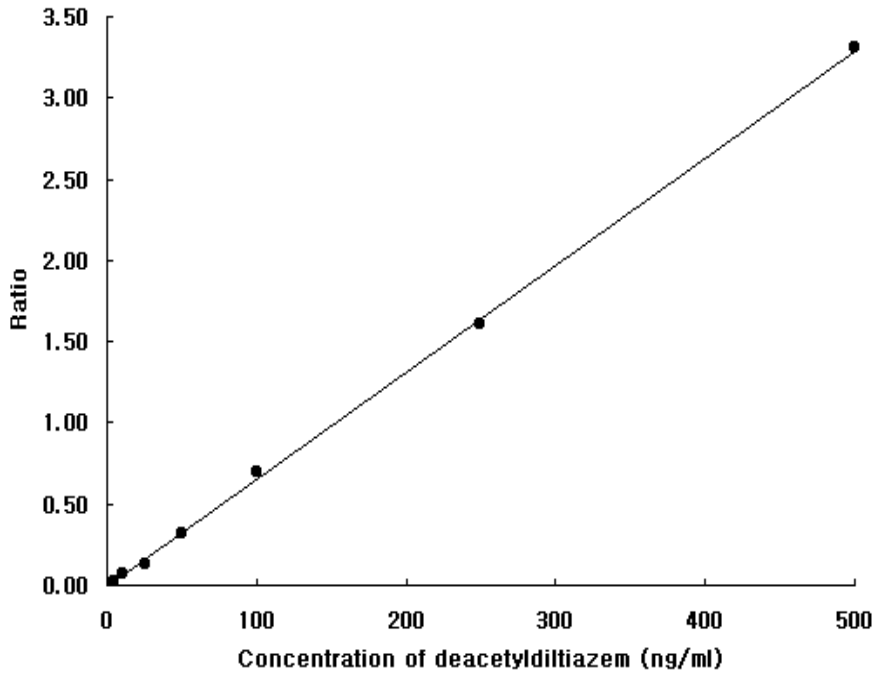

Figure 3. Calibration curve of desacetyldiltiazem spiked in rat plasma.

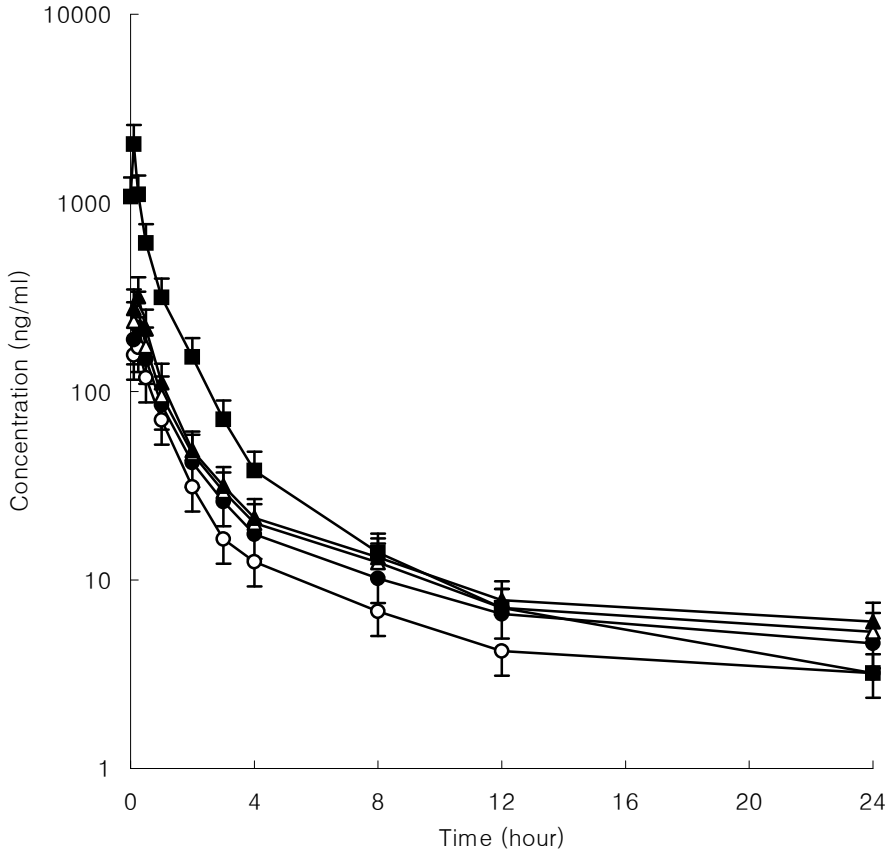

Figure 4. Mean plasma concentration-time profiles of diltiazem following an intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of fluvastatin (Mean ± SD, n = 6). ○; Control (diltiazem 15 mg/kg, oral), ●; coadministered with 0.5 mg/kg of , △; coadministered with 1.5 mg/kg of , ▲; coadministered with 3.0 mg/kg of , ■ ; i.v. injection of diltiazem (5 mg/kg).

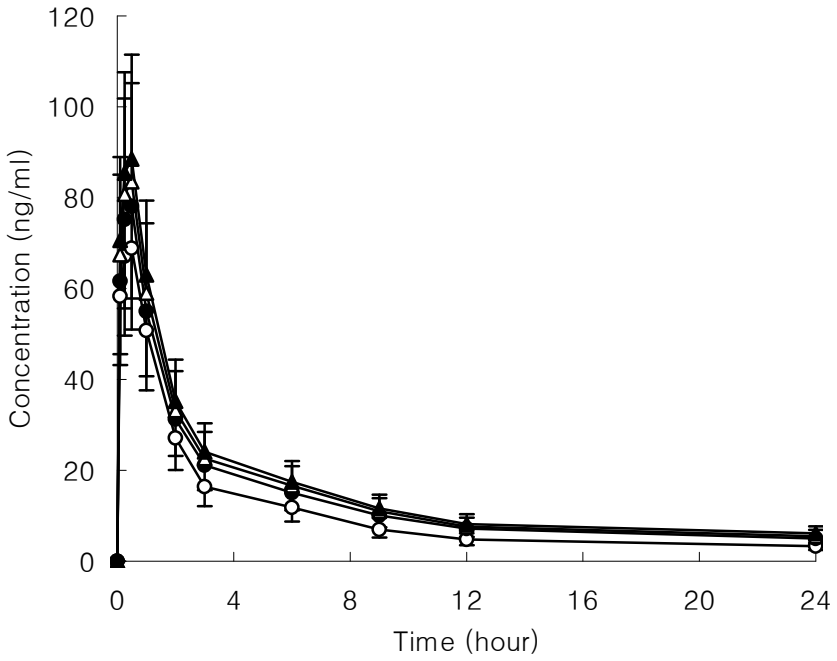

Figure 5. Mean plasma concentration-time profiles of desacetyldiltiazem after an oral administration of diltiazem (15 mg/kg) to rats in the presence and absence of fluvastatin (Mean ± SD, n = 6). ○; Control (diltiazem 15 mg/kg, oral), ●; coadministered with 0.5 mg/kg of , △; coadministered with 1.5 mg/kg of , ▲; coadministered with 3.0mg/kg of fluvastatin

Table 1. Mean plasma concentration of diltiazem after an intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of fluvastatin (Mean ±SD, n = 6)

Time (hour)	Control	fluvastatin 0.5mg/kg	fluvastatin 1.5mg/kg	fluvastatin 3.0mg/kg	I V
0	0	0	0	0	1080
0.1	156 ± 40.6	188 ± 48.9	236 ± 61.4	276 ± 71.8	2050
0.25	171 ± 44.5	218 ± 56.7	268 ± 69.7	319 ± 82.9	1109
0.5	118 ± 30.7	148 ± 38.5	173 ± 45.0	215 ± 55.9	612
1	70.5 ± 18.3	84.6 ± 22.0	95 ± 24.7	111.4 ± 29.0	315
2	31.2 ± 8.1	42 ± 10.9	46.7 ± 12.1	48.6 ± 12.6	152
3	16.5 ± 4.3	26 ± 6.8	29.5 ± 7.7	31.5 ± 8.2	71
4	12.5 ± 4.3	17.5 ± 4.6	20 ± 5.2	21.3 ± 5.5	38
8	6.8 ± 1.8	10.2 ± 2.7	12.4 ± 3.2	13.2 ± 3.4	14
12	4.2 ± 1.1	6.6 ± 1.7	7.1 ± 1.8	7.8 ± 2.0	7.1
24	3.2 ± 0.8	4.6 ± 1.2	5.3 ± 1.4	6 ± 1.6	3.2

Table 2. Mean plasma concentration of desacetyldiltiazem following an oral administration of diltiazem (15 mg/kg) to rats in the presence and absence of fluvastatin (Mean ±SD, n = 6)

Time (hour)	Control	fluvastatin 0.5mg/kg	fluvastatin 1.5mg/kg	fluvastatin 3.0mg/kg
0	0	0	0	0
0.1	58.3 ± 15.2	61.6 ± 16.0	67.5 ± 17.6	70.6 ± 18.4
0.25	67.1 ± 17.4	75.2 ± 19.6	80.8 ± 21.0	85.4 ± 22.2
0.5	68.9 ± 17.9	78.1 ± 20.3	83.5 ± 21.7	88.5 ± 23.0
1	50.8 ± 13.2	55 ± 14.3	59 ± 15.3	63 ± 16.4
2	27.1 ± 7.0	31.3 ± 8.1	33.2 ± 8.6	35.2 ± 9.2
3	16.4 ± 4.3	21.1 ± 5.5	22.6 ± 5.9	24.1 ± 6.3
6	11.8 ± 3.1	15.1 ± 3.9	16.6 ± 4.3	17.5 ± 4.6
9	7 ± 1.8	10.1 ± 2.6	11 ± 2.9	11.6 ± 3.0
12	4.8 ± 1.2	7.2 ± 1.9	7.6 ± 2.0	8.2 ± 2.1
24	3.3 ± 0.9	5 ± 1.3	5.5 ± 1.4	6.1 ± 1.6

Table 3. Mean pharmacokinetic parameters of diltiazem after an intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of fluvastatin (Mean ± SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + Fluvastatin			I.V. 5mg/kg
		0.5 mg/kg	1.5 mg/kg	3.0 mg/kg	
AUC (ng·hr/mL)	333 ± 83.3	491 ± 122.8*	642 ± 160.5*	738 ± 184.5**	1960 ± 490
C_{max} (ng/mL)	171 ± 42.8	220 ± 55*	374 ± 93.5*	410 ± 102.5**	-
T_{max} (hr)	0.25	0.25	0.25	0.25	-
K_a (hr^{-1})	2.8 ± 0.7	4.2 ± 1.1*	5.0 ± 1.3*	5.1 ± 1.3**	-
K_{e1}	0.064±0.02	0.062±0.02	0.060±0.02	0.059±0.01	-
$T_{1/2}$ (hr)	11.0 ± 2.8	11.2 ± 2.8	11.3 ± 2.8	11.7 ± 2.9	8.0 ± 1.5
A.B. (%)	6.1 ±1.5	8.4 ± 2.1*	11 ± 2.8*	13.2 ± 3.3**	-
R.B. (%)	100	136	155	177	-

Mean ± S.D. (n=6), * $p<0.05$, ** $p<0.01$, significant difference compared to the control (given diltiazem alone orally)

AUC: area under the plasma concentration-time curve from 0 hour th 24 hour

C_{max}: peak concentration

T_{max}: time to reach peak concentration

K_a: absorption rate constant

K_{el} : elimination rate constant

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

A.B. (%): absolute bioavailability

R.B. (%) : relative bioavailability compared to the control group

Table 4. Mean pharmacokinetic parameters of desacetyldiltiazem following an oral administration of diltiazem (15 mg/kg) to rats in the presence and absence of fluvastatin (Mean ±SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + Fluvastatin		
		0.5 mg/kg	1.5 mg/kg	3.0 mg/kg
AUC (ng ·hr/mL)	283 ± 70.8	382 ± 96.5*	406 ± 101.5*	448 ± 112.8*
C_{max} (ng/mL)	68.9 ± 17.2	78.1 ± 19.5	83.5 ± 20.9	88.5 ± 22.1*
T_{max} (hr)	0.5	0.5	0.5	0.5
$T_{1/2}$ (hr)	11.7± 2.9	13.5 ± 3.4	13.6 ± 3.4	14.1 ± 3.5
M.R.	0.85 ±0.21	0.85 ± 0.21	0.78 ± 0.20*	0.71 ± 0.18*
R.B. (%)	100	136	144	159

Mean ± S.D. (n=6), * $p<0.05$, significant difference compared to the control (given diltiazem alone orally)

AUC: area under the plasma concentration-time curve from 0 hour th 24 hour

C_{max}: peak concentration

T_{max}: time to reach peak concentration

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

M.R. (Metabolite-parent Ratio): ($AUC_{desacetyldiltiazem}/AUC_{diltiazem}$).

R.B. (%) : relative bioavailability compared to the control group

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