Pharmacokinetic interaction between diltiazem and atorvastatin in rats

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약 학 과

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이 논문을 약학 석사학위신청 논문으로 제출함.

2006 년 4 월 일

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Abstract

The present study aims to investigate the effect of atovastatin, HMG-CoA reductase inhibitor, on the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, in rats. Pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined after the oral administration of diltiazem (15 $mg \cdot kg^{-1}$) to rats in the presence or absence of atorvastatin (0.3, 1.0 or 3 $mg \cdot kg^{-1}$). Compared with the control group (given diltiazem alone), coadministration of atorvastatin significantly altered the pharmacokinetic parameters of diltiazem. The absorption rate constant (K_a), peak concentration (C_{max}) and the areas under the plasma concentration-time curve (AUC) of diltiazem were significantly increased in the presence of atovastatin. The AUC of diltiazem was increased by 1.47-fold in rats coadministered with 1.0 $mg \cdot kg^{-1}$ atorvastatin, and 1.75-fold with 3.0 $mg \cdot kg^{-1}$ atorvastatin, respectively, while there was no significant changes in time to reach the peak concentration (T_{max}) and terminal plasma half-life ($T_{1/2}$) of diltiazem. Consequently, absolute bioavailability values of diltiazem in rats coadministered with atorvastatin (7.9 to 11.6% in the presence of atorvastatin 0.3 to 3.0 $mg \cdot kg^{-1}$) were significantly higher ($p < 0.05$) than those from the control group (6.6%).

The presence of atorvastatin significantly ($p<0.05$) increased the AUC of desacetyldiltiazem. But metabolite-parent AUC ratio was significantly decreased by coadministration with atorvastatin (1.0-3.0 $mg \cdot kg^{-1}$), implying that atorvastatin could be effective to inhibit the metabolism of diltiazem. In conclusion, the concomitant use of atorvastatin significantly enhanced the oral bioavailability of diltiazem in rats.

Keyword: atorvastatin, desacetyldiltiazem, diltiazem, drug interaction, pharmacokinetic

국 문 초 록

아톨바스타틴과 딜티아젬의 약물동태학적 상호작용

김 현 용

지도교수 : 최준식

약 학 과

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고혈압 및 고지혈증 환자에서 아톨바스타틴과 딜티아젬의 병용처방이 가능하다. 그러므로 아톨바스타틴이 딜티아젬과 데스아세칠딜티아젬의 약물동태 파라미터에 영향을 미칠것으로 사료되어 딜티아젬 15 $mg \cdot kg^{-1}$ 과 아톨바스타틴 (0.3, 1.0, 3.0 $mg \cdot kg^{-1}$)을 흰쥐에 경구병용투 여하여 본 연 구를 실시하였다.

아톨바스타틴은 딜티아젬의 AUC, C_{max} 와 생체이용률을 유의성 있게 증가시켰다. 그 결과로 상대적생체이용률을 1.19-1.75 배 증가되었다. 아톨바스타틴은 딜 티아젬의 대 사체인 데스 아세칠딜티아젬의 약물 동태파라미터에 영향을 미 쳤다. 즉 데 스아세칠딜티아젬의 AUC 을 유의성 있게 증가시켰으며 MR (대사률)은 유의성 (1.0 와 3.0 $mg \cdot kg^{-1}$) 있게 감소 시켰다. 이것은 아톨바스타틴이 딜티아젬의 대사를 억제시킨 것으로 사료된다. 아톨바스타틴의 용량을 증가시킴과 더불어 딜티아젬의 생체이용률도 증가되었다. 따라서, 본 연구결과는 임상에서 아톨바스타틴과 딜티아젬을 병용투여시 딜티아젬의 용량를 조절 하는 것이 필요할수도 있 음을 시사 한다.

Introduction

Diltiazem is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension (Chaffman et al 1985; Weir 1995; Yeung et al 1993). Diltiazem undergoes the extensive and complex phase I metabolism including desacetylation, N-demethylation, and O-demethylation and its absolute bioavailability is approximately 40%, with a large inter-subject variability (Yeung et al 1993; Buckley et al 1990). In the preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appears to be about one half to equivalent compared with diltiazem, whereas the potencies of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem were about one third the potency of diltiazem (Narita et al 1986; Yeung et al 1998). Considering the potential contribution of active metabolites to the therapeutic outcome of diltiazem treatment, it could be important to monitor the active metabolites as well as the parent drug in the pharmacokinetic studies of diltiazem. CYP3A4, a key enzyme for the metabolism of diltiazem, is mainly located in liver, but it is also expressed in small intestine (Pichard et al 1990; Watkins et al 1987; Kolars et al 1992). Thus, diltiazem could be metabolized in small intestine as well as in liver (Lefebvre et al 1996; Homsy et al 1995a, b). Lee et al (1991) 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. In addition to the extensive metabolism, P-glycoprotein (P-gp) may also account for the low bioavailability of diltiazem. Yusa et al (1989) have reported that the calcium channel blockers such as verapamil, nicardipine and diltiazem competitively restrained the multi-drug resistance of P-gp. Wacher et al (2001) also suggested that diltiazem could be a substrate of both CYP 3A4 and P-gp. Since P-gp is co-localized with CYP3A4 in small intestine, P-gp and CYP3A4 may act synergistically for the presystemic drug metabolism, resulting in the limited absorption of drugs (Wacher et al 1998; 2001; Gottesman et al 1993; Gan et al 1996; Ito et al 1999).

Atorvastatin, hydroxymethylglutaryl-CoA(HMG-CoA) reductase inhibitor, is a lipid-lowering drug and also is used as an adjunct to dietary therapy to slow the progression of coronary atherosclerosis in hypercholestrolemic patients with coronary heart disease (Lea et al 1997). It is rapidly absorbed from the gastrointestinal tract. It shows low absolute bioavailability of about 12% due to presystemic clearance in the gastrointestinal mucosa and first-pass metabolism in the liver. Atorvastatin is mainly metabolized by the cytochrome p450 isoenzyme CYP3A4 to a number of active metaboltes (Lennernas 2003). Plasma protein binding exceeds 98 % (Lennernas 2003). The mean elimination half-life of atorvastatin is about 14 hours. Atorvastatin

is excreted as metaboltes, primatory in the bile (Lennernas 2003).

Antihypertensive drugs 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 cause increase in plasma concentrations of some statins, probably by inhibition of the cytochrome P450 isoenzyme CYP3A4-mediated metabolism. Pharmacokinetic studies have reported that plasma concentrations of simvastatin were increased in the presence of verapamil or diltiazem, and of lovastatin or pravastatin coadministered with diltiazem (Kantola et al 1998; Mousa et al 2000). The interaction between statins and diltiazem has also been reported in patients. 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 rhabdomyolysis resulting from the drug interaction between diltiazem and atorvastatin or simvastatin (Kanathur et al 2001; Peces et al 2001; Lewin et al 2002).

To further evaluate the pharmacokinetic interaction between calcium channel antagonist and HMG-CoA reductase, we would like to investigate the effect of atorvastatin on the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, in rats.

Materials and Methods

Materials

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and atorvastatin 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.

Animal Studies

All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) and the experimental protocols were approved by the Animal Care Committee of Chosun University. Male Sprague-Dawley rats (270-300g) were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and had free access to normal standard chow diet (Jae Ⅱ Chow, Korea) and tap water. Animals were kept in these facilities for at least one week before the experiment and fasted for 24 hrs prior to the experiments. Yeung et al (1990) reported that the oral administration of diltiazem to rats at 15 $mg \cdot kg^{-1}$ achieved the plasma level comparable to the

therapeutic concentrations in humans. Therefore, in the present study, rats (n=6 per each treatment) were given orally a 15 $mg \cdot kg^{-1}$ of diltiazem coadministered with atorvastatin (0.3, 1.0 or 3.0 $mg \cdot kg^{-1}$) and no concomitant treatment (diltiazem alone). Separately, 5 $mg \cdot kg^{-1}$ diltiazem was administered intravenously to rats. Blood samples were collected from the femoral artery at 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h post dose. Blood samples were centrifuged and the plasma was removed and stored at -40℃ until analyzed by HPLC.

HPLC Analysis

The plasma concentrations of diltiazem were measured using HPLC analysis system modified from the method of Goebel et al (1985). Briefly, 50 μL of imipramine (2 $\mu g \cdot mL^{-1}$), 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 at 5000 $rev \cdot min^{-1}$ for 10 min. One milliliter 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 $mL \cdot min^{-1}$ are as follows: internal standard at 10.5-min, diltiazem at 8.0-min and desacetyldiltiazem at 6.5-min. The calibration curves of diltiazem and desacetyldiltiazem were linear within the range of 10-400 $ng \cdot mL^{-1}$. 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. Recovery (%) assessed from the replicate analysis (n=5) for five days by adding 20 $ng \cdot mL^{-1}$ and 200 $ng \cdot mL^{-1}$ of diltiazem to the rat plasma was shown 106 ± 5.7 and 101 ± 4.9, respectively. Detection limit of diltiazem and desacetyldiltiazem was 10 $ng \cdot mL^{-1}$.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed by using Kinetica-4.3 (InnaPhase Corp., Philadelphia, PA, USA). 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 by $0.693/K_{el}$. The absolute bioavailability (AB%) of diltiazem was calculated by $AUC_{oral}/AUC_{iv} \times Dose_{i.v}/Dose_{oral} \times 100$, and the relative bioavailability (RB%) of diltiazem was estimated by $AUC_{coadjmin}$ / $AUC_{control}$ × 100. The metabolite-parent ratio (MR) was estimated by ($AUC_{desacetyldiltiazem}$ /$AUC_{diltiazem}$) × (M.W. $_{diltiazem}$/M.W. $_{desacetyldiltiazem}$).

Statistical analysis

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a 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 atorvastatin were characterized in rats and illustrated in Fig.4. The mean pharmacokinetic parameters of diltiazem were also summarized in Table 1.

As shown in Table 3, the coadministration of atorvastatin (0.3, 1.0 or 3.0 $mg \cdot kg^{-1}$) significantly altered the pharmacokinetic parameters of diltiazem compared to the control given diltiazem alone. The C_{max}, and AUC of diltiazem were significantly increased in the rats coadministered with atorvastatin ($p<0.05$, 0.3 or 1.0 $mg \cdot kg^{-1}$; $p< 0.01$, 3.0 $mg \cdot kg^{-1}$, respectively), while there was no significant change in T_{max} and terminal plasma half-life ($T_{1/2}$) of diltiazem in the presence of atorvastatin (Table 3). Consequently, absolute bioavailability values (AB%) of diltiazem in the rats coadministered with atorvastatin were significantly higher ($p<0.05$, 0.3 or 1.0 $mg \cdot kg^{-1}$; $p< 0.01$, 3.0 $mg \cdot kg^{-1}$, respectively) than those from the control group. Atorvastatin caused increases in the mean plasma concentration of oral administered diltiazem in rats in dose-dependent manner over the dose range of 0.3 to 3.0 $mg \cdot kg^{-1}$. The pharmacokinetic profiles of desacetyldiltiazem, an active metabolite of diltiazem, were also evaluated in the presence and absence of atorvastatin (Fig. 5). As summarized in Table 4, the oral exposure

of desacetyldiltiazem in the rats coadministered with atorvastatin caused significant increase in AUC ($p<0.05$). However, the metabolite-parent ratio (M.R.) in the presence of 1.0 or 3.0 $mg \cdot kg^{-1}$ of atorvastatin decreased significantly ($p<0.05$) compared to the control group.

Taken together, coadministration of atorvastatin significantly enhanced the oral exposure of diltiazem in rats.

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, CYP3A4 and P-gp have been recognized as a concerted barrier to the drug absorption (Benet et al 2003; Cummins et al 2002).Therefore, dual inhibitors against both CYP3A4 and P-gp could have a great impact on the bioavailability of many drugs where CYP3A4 metabolism as well as P-gp mediated efflux is the major barrier to the systemic availability. Besides the extensive metabolism by CYP3A4, diltiazem appeared to be the substrate of P-gp, suggesting that P-gp and CYP3A4 could act synergistically to limit the oral bioavailability of diltiazem (Wacher et al 2001; Saeki et al 1993). Clinically, antihypertensive agents are commonly coadministered with cholesterol-lowering agents. There are some reports about the effect of calcium channel antagonists on the pharmacokinetc parameters of HMG-CoA reductase inhibitors. However, there is less information about the effect of atorvastatin, HMG-CoA reductase inhibitors, on the pharmacokinetcs of diltiazem, calcium channel antagonists, and its main metabolite, desacetyldiltiazem. Therefore, more preclinical and clinical investigations on the atorvastatin-diltiazem interaction should be performed to prevent potential adverse reactions or to utilize those

interactions for a therapeutic benefit. The present study evaluated the effect of atorvastatin on the pharmacokinetics of diltiazem in rats to examine a potential drug interaction between atorvastatin and diltiazem which are substrates or inhibitors of CYP3A4 and P-gp (Kantola et al 1998; Wu et al 2000; Boyd et al 2000; Lilja et al 1999; Bogman et al 2001; Siedlik et al 1999; Renders et al 2001). As shown in Table 1, the presence of atorvastatin significantly enhanced the C_{max}, AUC and K_a of oral-administered diltiazem, which might be due to the inhibition of CYP3A4 and P-gp by atorvastatin (Boyd et al 2000; Bogman et al 2001; Renders et al 2001; Wang et al 2001). Subsequently, relative bioavailability of diltiazem was increased by 147 to 175% in the rats coadministered with atorvastatin (1.0 to 3.0 $mg \cdot kg^{-1}$). These results are similar to the observation from the previous studies, in that atorvastatin increased blood concentration of cyclosporin, a substrate of P-gp and CYP3A4 and decreased the total plasma clearance of midazolam (Renders et al 2001; McDonnell et al 2003). And also, results from the present study are consistent with the previous studies reported by Boyd et al (2000). In their studies, atorvastatin has been shown to affect pharmacokinetics of a well-known P-gp substrate, digoxin, with C_{max} and AUC increasing by 20%. In the previous studies, atorvastatin, lovastatin and simvastatin inhibited rhodamine 123 (a P-gp probe) transport in a murine monocytic leukaemia cell line overexpressing the transporter, which were

very potent and effective inhibitors of P-gp-mediated transport (Bogman et al 2001; Wang et al 2001). This study showed that atorvastatin over the dose range of 1.0 $mg \cdot kg^{-1}$ to 3.0 $mg \cdot kg^{-1}$ was effective to enhance the oral exposure of P-gp substrates as well as CYP3A4 substrates in dose-dependent manner. The pharmacokinetic profiles of desacetyldiltiazem were also evaluated in the presence and absence of atorvastatin (Table 2). The metabolite-parent ratio in the rats coadministered with 1.0-3.0 $mg \cdot kg^{-1}$ of atorvastatin decreased significantly ($P<0.05$) compared to the control, implying that the presence of atorvastatin could be effective to inhibit presystemic metabolism of diltiazem. Lee et al (1991) 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. Therefore, the decrease of intestinal extraction by the concomitant use of atorvastatin resulted in the enhanced oral bioavailability of diltiazem. The concomitant use of atorvastatin increased the C_{max} and AUC of diltiazem with decreasing the metabolite-parent ratio. Those results suggest that atorvastatin could reduce the metabolism in the GI tract during the intestinal absorption. Taken all together, the pharmacokinetics of diltiazem was significantly altered by the coadministration of atorvastatin in rats. So far pharmacokinetic data of drugs with atorvastatin are scarce, particularly in humans and it is

hard to discuss the clinical dose of atorvastatin over the therapeutic concentration of diltiazem at this moment. Since the present study raised the awareness about the potential drug interactions by concomitant use of atorvastatin with diltiazem, the clinical significance of this finding need to be further evaluated in the clinical studies.

Conclusion

Coadministration of atorvastatin over the dose range of 1 $mg \cdot kg^{-1}$ to 3 $mg \cdot kg^{-1}$ significantly enhanced the oral bioavailability of diltiazem. Therefore, concomitant use of diltiazem with atorvastatin may require close monitoring for potential drug interactions.

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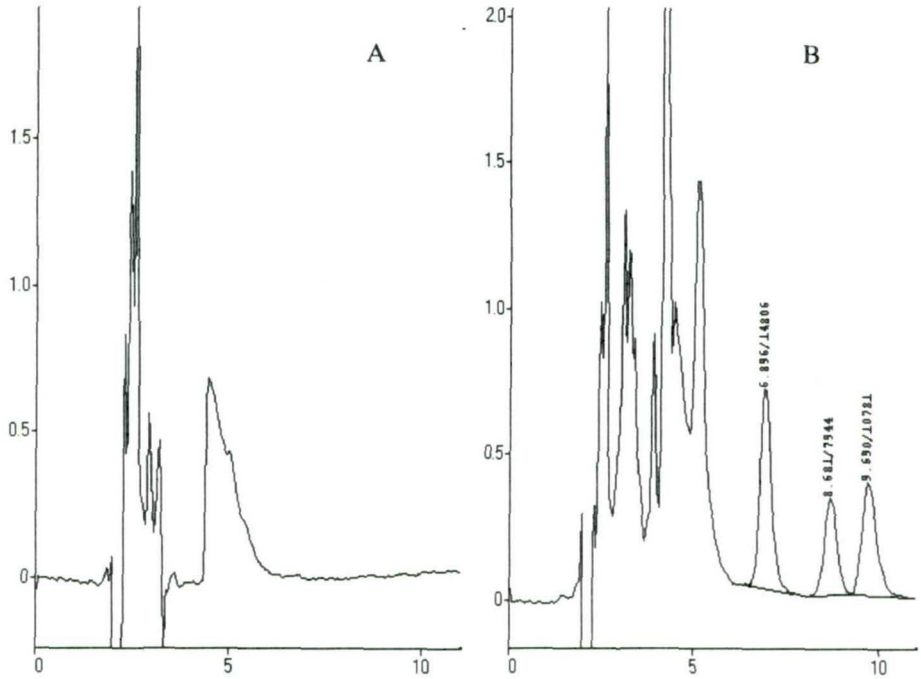

Figure 1. Chromatogram of rat's blank plasma (A) and the plasma (B) spiked with desacetyldiltiazem (6.9 min), diltiazem (8.7 min) and the internal stand, imipramine (9.7 min).

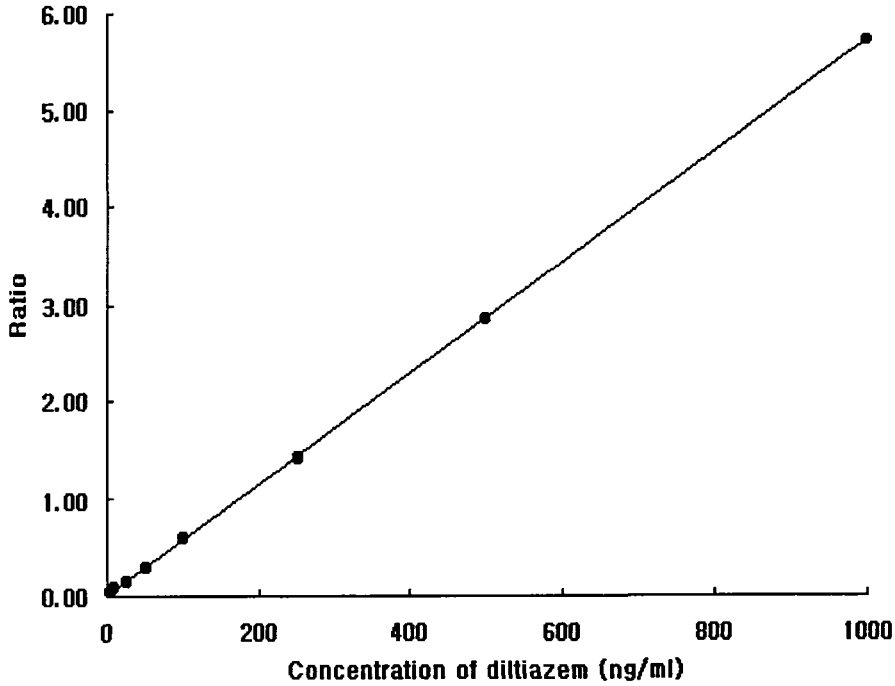

Figure 2. Calibration curve of diltiazem spiked in rat plasma.

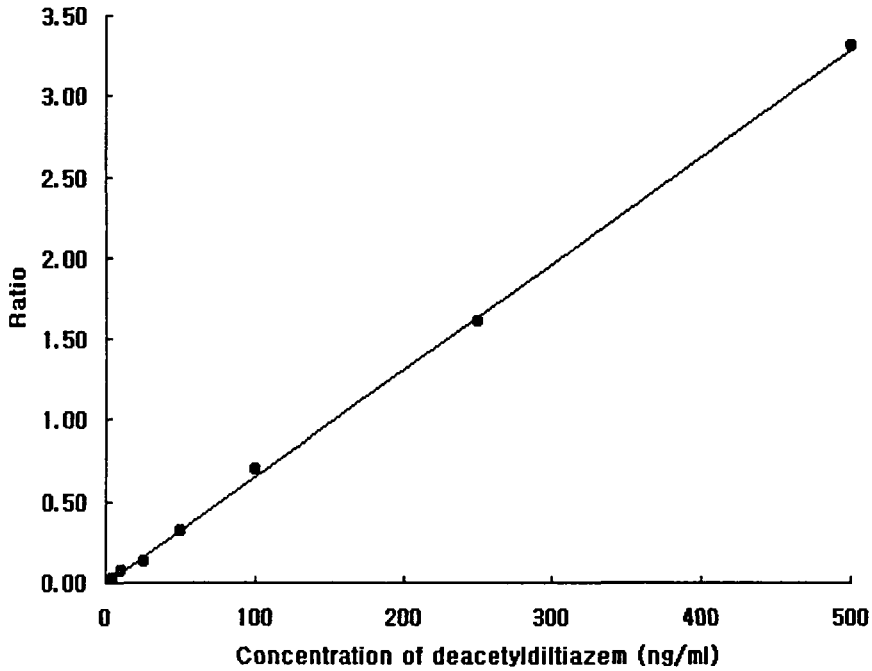

Figure 3. Calibration curve of desacetyldiltiazem spiked in rat plasma.

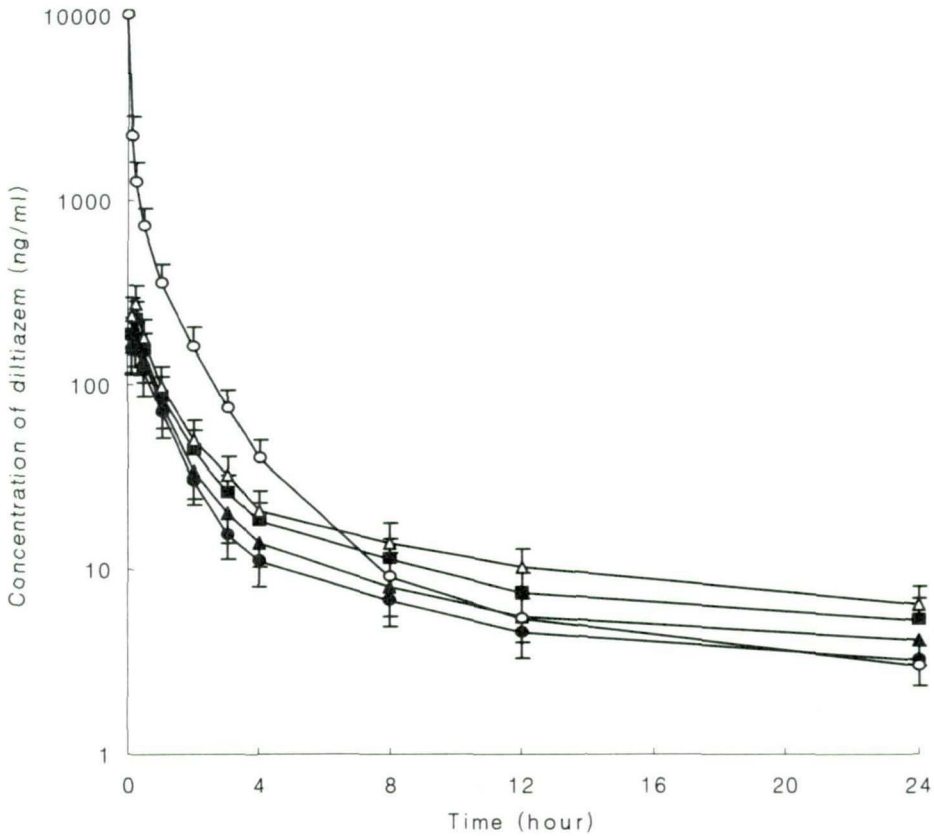

Figure 4. Mean plasma concentration-time profiles of diltiazem following an intravenous (5 mg·kg^{-1}) or oral (15 mg·kg^{-1}) administration of diltiazem to rats in the presence and absence of atorvastatin (Mean + SD, n = 6). ●; Control (diltiazem 15 mg·kg^{-1}, oral), ▲; coadministered with 0.3 mg·kg^{-1} of atovastatin, ■; coadministered with 1.0 mg·kg^{-1} of atorvastatin, △; coadministered with 3.0 mg·kg^{-1} of atorvastatin, ○; i.v. injection of diltiazem (5 mg·kg^{-1}).

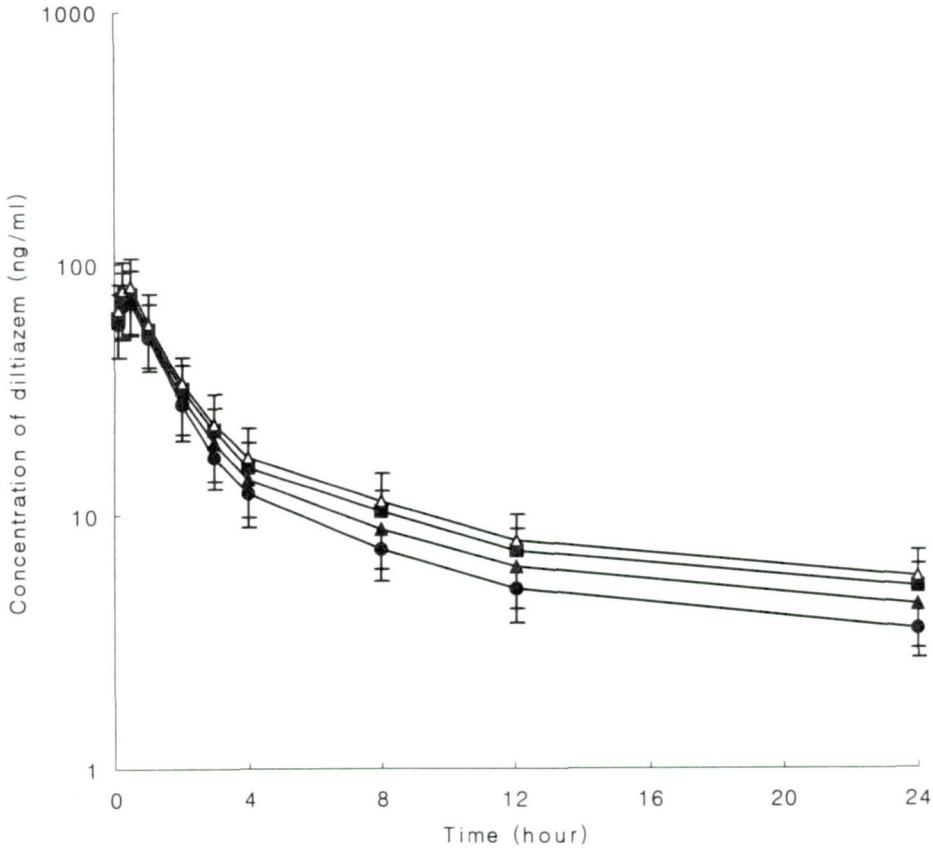

Figure 5. Mean plasma concentration-time profiles of desacetyldiltiazem after an oral administration of diltiazem (15 mg·kg^{-1}) to rats in the presence and absence of atorvastatin (Mean + SD, n = 6). ●; Control (diltiazem 15 mg·kg^{-1}, oral), ▲; coadministered with 0.3 mg·kg^{-1} of atovastatin, ■; coadministered with 1.0 mg·kg^{-1} of atorvastatin, △; coadministered with 3.0 mg·kg^{-1} of atorvastatin.

Table 1. Mean plasma concentration of diltiazem after an intravenous (5 $mg \cdot kg^{-1}$) or oral (15 $mg \cdot kg^{-1}$) administration of diltiazem to rats in the presence and absence of atrovastatin (Mean ±SD, n = 6)

Time (hour)	Control	atrovastatin 0.3 $mg \cdot kg^{-1}$	atrovastatin 1.0 $mg \cdot kg^{-1}$	atrovastatin 3.0 $mg \cdot kg^{-1}$	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 $mg \cdot kg^{-1}$) to rats in the presence and absence of atrovastatin (Mean ±SD, n = 6)

Time (hour)	Control	atrovastatin 0.3 $mg \cdot kg^{-1}$	atrovastatin 1.0 $mg \cdot kg^{-1}$	atrovastatin 3.0 $mg \cdot kg^{-1}$
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 $mg \cdot kg^{-1}$) or oral (15 $mg \cdot kg^{-1}$) administration of diltiazem to rats in the presence and absence of atrovastatin (Mean ±SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + Atrovastatin			I.V. 5 $mg \cdot kg^{-1}$
		0.3 $mg \cdot kg^{-1}$	1.0 $mg \cdot kg^{-1}$	3.0 $mg \cdot kg^{-1}$	
AUC ($ng \cdot hr \cdot mL^{-1}$)	337 ± 58.6	402 ± 85.8*	496 ± 106.2*	587 ± 114.8**	1692 ± 406
C_{max} ($ng \cdot mL^{-1}$)	170 ± 41.9	195 ± 54.9*	223 ± 56.6*	272 ± 67.2**	-
T_{max} (hr)	0.25	0.25	0.25	0.25	-
$T_{1/2}$ (hr)	12.1 ± 2.9	12.4 ± 3.6	12.6 ± 3.6	12.7 ± 3.7	6.4 ± 1.7
A.B. (%)	6.6 ±1.5	7.9 ± 1.9*	9.8 ± 2.5*	11.6 ± 2.7**	-
R.B. (%)	100	119	147	175	-

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

$t_{1/2}$: 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 $mg \cdot kg^{-1}$) to rats in the presence and absence of atrovastatin (Mean ±SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + Atrovastatin		
		0.3 $mg \cdot kg^{-1}$	1.0 $mg \cdot kg^{-1}$	3.0 $mg \cdot kg^{-1}$
AUC ($ng \cdot hr \cdot mL^{-1}$)	295 ± 75.4	343 ± 86.1*	389 ± 96*	421 ± 102*
C_{max} ($ng \cdot mL^{-1}$)	70 ± 16.8	73 ± 17.2	76 ± 18.0	82 ± 18.6*
T_{max} (hr)	0.5	0.5	0.5	0.5
$T_{1/2}$ (hr)	12.4± 3.1	13.5 ± 3.4	13.6 ± 3.4	14.1 ± 3.5
M.R.	0.87 ±0.21	0.85 ± 0.23	0.77 ± 0.16*	0.73 ± 0.15*

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): ($AUC_{desacetyldiltiazem}/AUC_{diltiazem}$).

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