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Short communication

Effects of simvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, after oral and intravenous administration in rats: possible role of P-glycoprotein and CYP3A4 inhibition by simvastatin

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Abstract:
The purpose of this study was to investigate the possible effects of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, simvastatin, on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats. HMG-CoA reductase inhibitors and diltiazem are sometimes prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. The effect of simvastatin on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activity was evaluated. Simvastatin inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC_{50}) of 3.0 μM. In addition, simvastatin significantly enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp. The pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined after oral and intravenous administration of diltiazem to rats in the presence and absence of simvastatin (0.3 and 1.0 mg/kg). The areas under the plasma concentration-time curve (AUC) and the peak concentration (C_{max}) of diltiazem were significantly ($p < 0.05$, 1.0 mg/kg) increased by 45.2% and 35.2%, respectively, in the presence of simvastatin compared to control. Consequently, the absolute bioavailability (AB) values of diltiazem in the presence of simvastatin (1.0 mg/kg) were significantly ($p < 0.05$) higher (44.8%) than that of the control group. Moreover, the relative bioavailability (RB) of diltiazem was 1.21- to 1.45-fold greater than that in the control group. The metabolite-parent AUC ratio (MR) in the presence of simvastatin (1.0 mg/kg) significantly decreased compared to the control group. This result implied that simvastatin effectively inhibited the metabolism of diltiazem.
The increase in diltiazem oral bioavailability might be attributable to enhanced absorption in the small intestine *via* the inhibition of P-gp and to reduced first-pass metabolism of diltiazem *via* the inhibition of the CYP3A subfamily in the small intestine and/or in the liver rather than renal elimination of diltiazem by simvastatin.

Key words:
diltiazem, desacetyldiltiazem, simvastatin, CYP3A, P-gp, pharmacokinetics, bioavailability, rats

Abbreviations: AB (%)– absolute bioavailability, AUC – area under the plasma concentration-time curve, C_{max} – peak plasma concentration, K_a – absorption rate constant for the diltiazem, K_{12} – distribution rate constant of the drug from the central compartment to the peripheral compartment, K_{21} – distribution rate constant from the peripheral compartment to the central compartment, MR – metabolite-parent ratio, RB (%) – relative bioavailability, $t_{1/2}$ – terminal half-life, T_{max} – time to peak concentration.

Introduction

Diltiazem is a Ca^{2+} channel blocker widely used in the treatment of angina, supraventricular arrhythmias and hypertension [6, 37, 39]. 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 a large inter-subject variability [5, 39]. In preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one half to equivalent compared to diltiazem, whereas the potencies of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem were about one third the potency of diltiazem [29, 38]. 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 the pharmacokinetic studies of diltiazem.

Cytochrome P450 (CYP) 3A4, a key enzyme in the metabolism of diltiazem, is mainly localized in the liver but is also expressed in the small intestine [1, 20, 30, 36]. Thus, diltiazem could be metabolized in the small intestine as well as the liver [15, 16, 22]. Lee et al. [21] reported that the extraction ratios of diltiazem in the small intestine and liver after oral administration to rats were about 85% and 63%, respectively [21]. This suggested that diltiazem was highly extracted in both organs. In addition to the extensive metabolism, P-glycoprotein (P-gp) may also account for the low bioavailability of diltiazem. Yusa and Tsuruo [40] reported that calcium channel blockers such as verapamil and diltiazem competitively restrained the multi-drug resistance of P-gp [40]. Wacher et al. [33] also suggested that diltiazem could act as a substrate of both CYP3A4 and P-gp [11, 13, 18, 33, 34]. Because P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism, resulting in the limited absorption.

Simvastatin, a HMG-CoA reductase inhibitor, lowers plasma low-density lipoprotein (LDL) levels and is widely used in treating hypercholesterolemia which is one of the risk factors of patients suffering from cardiovascular diseases such as ischemic heart disease and hypertension. Simvastatin is rapidly absorbed from the gastrointestinal tract after oral administration but undergoes extensive first-pass metabolism in the liver [23]. Tubic-Grozdanis et al. [32] reported that simvastatin is often administered as a lactone and is metabolically activated to the open chain nonlactone simvastatin acid. This reversible and active conversion occurs by nonspecific carboxyesterases in the intestinal wall, liver and to some extent in plasma or by nonenzymatic hydrolysis [32]. The oxidative biotransformation of simvastatin is primarily mediated by CYP3A4 [4, 31], and the Center for Drug Evaluation and Research of the Food and Drug Administration recently recommended simvastatin as a probe drug when studying the potential of different drugs to inhibit or induce CYP3A4 *in vivo* in humans [10] and as an inhibitor of P-gp [3]. However, the effect of simvastatin on the inhibition of CYP3A4 and P-gp activity is somewhat ambiguous. Thus, we attempted to evaluate P-gp activity using the rhodamine-123 retention assay in P-gp-overexpressing MCF-7/ADR cells, and assessed CYP3A4 activity.

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. Because it is a co-substrate of CYP3A4 and P-gp, orally administered simvastatin would affect the pharmacokinetics and metabolism of diltiazem.

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 increase the plasma concentrations of some statins, possibly through the inhibition of CYP3A4 and P-gp [28]. But there are few reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [7, 17]. Moreover, simvastatin and diltiazem could be prescribed for the prevention or treatment of cardiovascular diseases as a combination therapy. Thus, the purpose of this study was to investigate the possible effects of sim-

vastatin on CYP3A4 and P-gp activity and bioavailability or the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, after oral and intravenous administration of diltiazem with simvastatin in rats.

Materials and Methods

Materials

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and simvastatin were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Acetonitrile, methanol, and *tert*-butylmethylether were from Merck Co. (Darmstadt, Germany). Rhodamine was from Calbiochem (San Diego, CA, USA), and the CYP inhibition assay kit was from GENTEST (Woburn, MA, USA). 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 tap water and a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea). Throughout the experiments, the animals were housed, 4 or 5 per cage, in laminar flow cages maintained at 22 ± 2°C with 50–60% relative humidity, under a 12 h light-dark cycle. The rats were acclimated to 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 (*iv*) 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 diethyl ether anesthesia.

Intravenous and oral administration of diltiazem

The rats were divided into nine groups (n = 6, each): oral groups [12 mg/kg of diltiazem dissolved in distilled 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 intravenous groups (4 mg/kg of diltiazem; the same solution used: 0.9% NaCl-injectable solution; total injection volume of 1.5 ml/kg) without (control) or with 0.3 and 1.0 mg/kg of simvastatin. A feeding 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 heparinized tubes *via* the femoral artery at 0 (control), 0.017 (at the end of infusion), 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h for the *iv* study and 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 loss due to repeated blood sampling. The blood samples were centrifuged (13,000 rpm, 3 min), and a 200-μL aliquot of plasma samples was stored at –40°C until the HPLC analysis.

HPLC assay

The plasma concentrations of diltiazem were determined using a modified version of the HPLC assay reported by Goebel and Kolle [12]. Briefly, 50 μl of imipramine (2 μg/ml), as the internal standard, and 1.2 ml of *tert*-butylmethylether was added to 0.2 ml of the plasma sample. It was then vortexed for 2 min and centrifuged at 13,000 rpm for 10 min. The organic layer (1 ml) was transferred to another test tube, and 0.2 ml of 0.01 M hydrochloric acid was added and mixed for 2 min. The water layer (50 μL) was injected into an HPLC system. The detector wavelength was set at 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 limits of diltiazem and desacetyldiltiazem in rat plasma were all 5 ng/ml. The coefficients of variation for diltiazem and desacetyldiltiazem were all below 5.0%.

CYP3A4 inhibition assay

The assay of inhibition assay was performed in a multi-well plate using CYP inhibition assay kit (GENTEST, Woburn, MA, USA) as described previously [8]. Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. CYP substrate (7-BFC for CYP3A4) was incubated with or without test compounds in the enzyme/substrate buffer with 1 pmol of P450 enzyme and an NADPH-generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution 45 min after incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA, USA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. A positive control (1 μM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. All experiments were done in duplicate, and the results were expressed as the percent of inhibition.

Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a reported method [14]. MCF-7/ADR cells were 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. Then, the culture medium was changed to Hanks' balanced salt solution, and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence or absence of simvastatin (1, 3 and 10 μM) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in EBC lysis buffer. Rhodamine-123 fluorescence in the resulting 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 are presented as the ratio to control.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated assuming a two-compartment open model by a nonlinear least-square regression analysis using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, 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 $AUC_{oral}/AUC_{iv} \times dose_{iv}/dose_{oral}$, 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 $AUC_{desacetyldilitiazem}/AUC_{diltiazem}$.

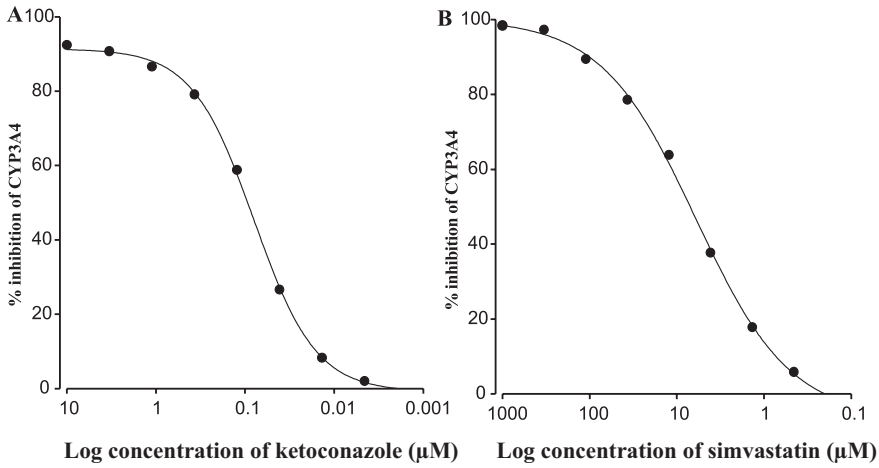

Fig. 1. Inhibitory effect of ketoconazole (**A**) and simvastatin (**B**) on CYP3A4 activity. All experiments were done in duplicate, and the results are expressed as the percent of inhibition

Statistical analysis

Results are presented as the means ± standard deviation (SD). The pharmacokinetic parameters were compared using one-way analysis of variance (ANOVA), followed by a *post-hoc* Dunnett's corrections. A p value < 0.05 was considered statistically significant.

Results

Inhibition of CYP3A4

The inhibitory effect of simvastatin on CYP3A4 activity is shown in Figure 1. Simvastatin inhibited CYP3A4 activity in a concentration-dependent manner with an IC_{50} value of 3.0 μM.

Rhodamine-123 retention assay

Accumulation of rhodamine-123, a P-gp substrate, was increased in MCF-7/ADR cells overexpressing P-gp compared with MCF-7 cells lacking P-gp, as shown in Figure 2. Simvastatin (1–10 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner. This result suggests that simvastatin significantly inhibited P-gp activity.

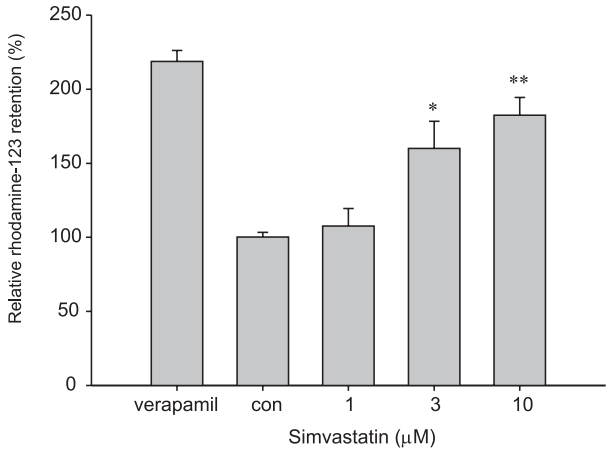

Fig. 2. Effects of simvastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents the mean ± SD (n = 6). * p < 0.05, ** p < 0.01, significant difference compared to positive control (verapamil)

Tab. 1. Mean pharmacokinetic parameters of diltiazem after oral (12 mg/kg) administration of diltiazem in the presence and absence of simvastatin in rats

Pharmacokinetic parameters	Diltiazem (control)	Diltiazem and simvastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng•h/ml)	270 ± 51	328 ± 62	392 ± 74*
C_{max} (ng/ml)	182 ± 33	217 ± 39	246 ± 44*
$t_{1/2\,(\beta)}$ (h)	5.6 ± 1.2	5.7 ± 1.2	5.8 ± 1.3
T_{max} (h)	0.21 ± 0.04	0.24 ± 0.05	0.24 ± 0.05
Ka (h^{-1})	47.4 ± 9.0	47.4 ± 9.0	47.3 ± 8.9
K_{12} (h^{-1})	0.77 ± 0.15	0.87 ± 0.17	0.94 ± 19
K_{21} (h^{-1})	0.44 ± 0.09	0.51 ± 0.11	0.52 ± 0.12
AB (%)	6.7 ± 1.2	8.1 ± 1.5	9.7 ± 1.8*
RB (%)	100	121	145

Values are the mean ± SD, (n = 6);* p < 0.05 (significant difference compared to the control)

Effect of simvastatin on the pharmacokinetics of oral diltiazem

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 3. The pharma-

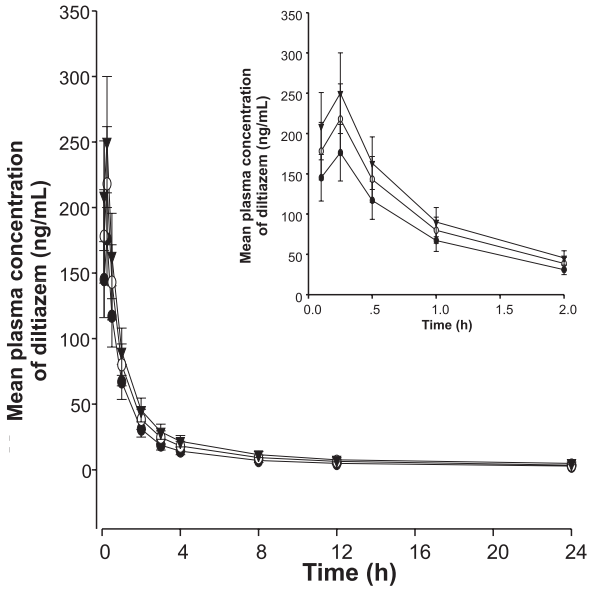

Fig. 3. Mean plasma concentration-time profiles of diltiazem after oral (12 mg/kg) administration of diltiazem in the presence and absence of simvastatin to rats (the mean ± SD, n = 6). • – Control (diltiazem 12 mg/kg), o – with 0.3 mg/kg simvastatin, ▼ – with 1.0 mg/kg simvastatin

cokinetic parameters of diltiazem are summarized in Table 1. Simvastatin (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 45.2% and increased the peak concentration (C_{max}) of diltiazem by 35.2%. Accordingly, the absolute bioavailability (AB) values of diltiazem in the presence of simvastatin (1.0 mg/kg) were significantly ($p < 0.05$) higher (44.8%) than that of the control group. Simvastatin increased the relative bioavailability (RB) of diltiazem 1.21- to 1.45-fold. The terminal half-life ($t_{1/2}$) of diltiazem was significantly ($p < 0.05$) prolonged, but the time to reach peak concentration (T_{max}) was unchanged. The K_a, K_{12}, and K_{21} values of diltiazem were not statistically significant.

Effect of simvastatin on desacetyldiltiazem pharmacokinetics

The plasma concentration-time profiles of desacetyldiltiazem are shown in Figure 4. The pharmacokinetic parameters of desacetyldiltiazem are summarized in Table 2. The metabolite-parent ratios (MRs) were significantly ($p < 0.05$, 1.0 mg/kg) decreased (24.1%) by simvastatin, suggesting that it affected deacetyldiltiazem formation. Thus, the increased bioavailability of diltiazem by simvastatin may be due both to its the inhibition of P-gp activity in the small intestine and its the inhibition of CYP3A4 activity in the small intestine and/or in the liver.

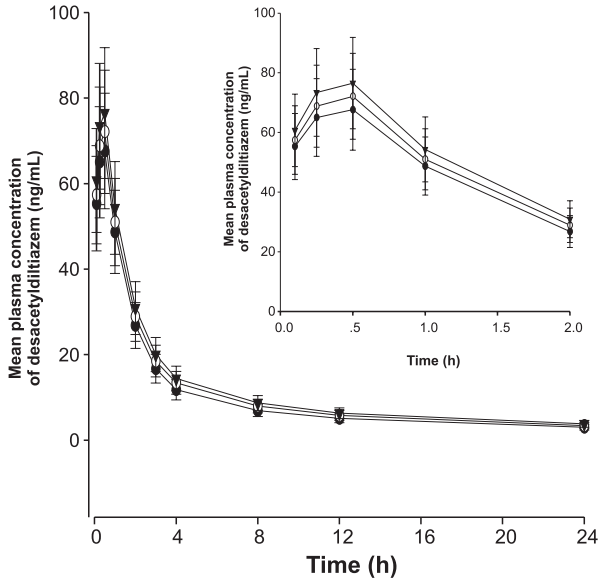

Fig. 4. Mean plasma concentration-time profiles of desacetyldiltiazem after oral administration of diltiazem (12 mg/kg) in the presence and absence of simvastatin to rats (the mean ± SD, n = 6). • – Control (diltiazem 12 mg/kg), o – with 0.3 mg/kg simvastatin, ▼ – with 1.0 mg/kg simvastatin

Tab. 2. Mean pharmacokinetic parameters of desacetyldiltiazem following oral administration of diltiazem (12 mg/kg) in the presence and absence of simvastatin in rats

Parameters	Diltiazem (control)	Diltiazem and simvastatin	
		0.3 mg/kg	1.0 mg/kg
AUC (ng•h/ml)	280 ± 53	301 ± 57	325 ± 62
C_{max} (ng/ml)	66.9 ± 12.1	71.4 ± 12.9	76.1 ± 13.7
T_{max} (h)	0.46 ± 0.12	0.44 ± 0.10	0.43 ± 0.10
$t_{1/2\,(\beta)}$ (h)	9.2 ± 1.7	10.2 ± 1.9	10.6 ± 2.0
K_{12} (h^{-1})	0.44 ± 0.09	0.46 ± 0.13	0.46 ± 0.13
K_{21} (h^{-1})	0.16 ± 0.03	0.18 ± 0.04	0.18 ± 0.04
MR	1.03 ± 0.22	0.92 ± 0.18	0.83 ± 0.14*

Values are the mean ± SD, (n = 6);* $p < 0.05$ (significant difference compared to the control)

Effect of simvastatin on the pharmacokinetics of *iv* diltiazem

Mean arterial plasma concentration-time profiles of diltiazem following *iv* administration of diltiazem (4 mg/kg) to rats in the presence or absence of simvastatin (0.3 and 1.0 mg/kg) are shown in Figure 5, the corresponding pharmacokinetic parameters are shown in Table 3. The AUC of diltiazem was increased, but was not statistically significant compared to control. The $t_{1/2}$ of diltiazem was also prolonged but

Tab. 3. Mean (± SD) pharmacokinetic parameters of diltiazem after intravenous (4 mg/kg) administration of diltiazem with simvastatin in rats

Parameters	Diltiazem (control)	Diltiazem + simvastatin	
		0.3 mg/kg	1 mg/kg
AUC (ng•h/ml)	1354 ± 230	1504 ± 256	1590 ± 270
CL_t (ml/min)	41.2 ± 7.4	38.0 ± 6.8	36.1 ± 6.5
$t_{1/2\,(\beta)}$ (h)	6.9 ± 1.2	7.1 ± 1.3	7.2 ± 1.4

Mean ± SD (n = 6). AUC: area under the plasma concentration–time curve from zero to infinity, CL_t: total body clearance, $t_{1/2}$: terminal half-life

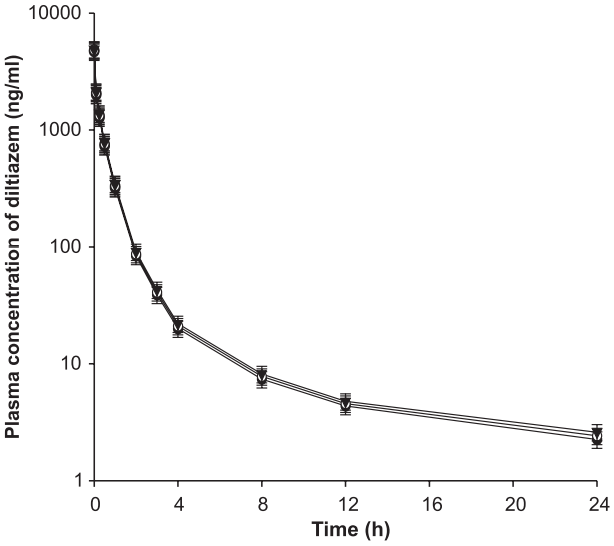

Fig. 5. Mean plasma concentration-time profiles of diltiazem after intravenous (4 mg/kg) administration of diltiazem in the presence and absence of simvastatin to rats (the mean ± SD, n = 6). • – Control (diltiazem 12 mg/kg), o – with 0.3 mg/kg simvastatin, ▼– with 1.0 mg/kg simvastatin

not significantly. In contrast to oral diltiazem, the pharmacokinetics of *iv* diltiazem were not affected by the concurrent use of simvastatin. Accordingly, the enhanced oral bioavailability in the presence of simvastatin may be mainly due to inhibition of CYP3A-mediated metabolism of diltiazem in the small intestine and/or in the liver by simvastatin rather than renal elimination of diltiazem.

Discussion

Intestinal 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. Using functional enzyme activity studies and immunoblot analyses, it was shown that CYP3A expression in mature enterocytes of jejunal mucosa is primarily located in the villi tipsand and is comparable or even greater than hepatocyte expression of CYP3A [35]. Total CYP P450 content increased slightly proceeding from the duodenum to the jejunum and then decreased sharply in the ileum [41]. Using *in situ* hybridization with a probe specific for CYP3A4, McKinnon and McManus confirmed CYP3A expression throughout the entire small intestine, with the highest levels in proximal regions [27]. The most abundant CYP isoenzymes in the intestine is 3A4 [32].

Based on their broad overlap in substrate specificities and their co-localization in the small intestine, which is the primary site of absorption for orally administered drugs, CYP3A4 and P-gp have been recognized as a concerted barrier to drug absorption [9, 34]. The prescription of more than one drug as part of 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 [26].

Considering that the drugs used in combination therapy often share the same metabolic pathways or cellular transport mechanisms, there is a high potential for pharmacokinetic and pharmacodynamic drug interactions between calcium channel antagonists and HMG-CoA reductase inhibitors. Indeed, some studies have reported that calcium-channel blockers increased the plasma concentration of simvastatin [28]. Similarly, as substrates of both CYP3A and P-gp, diltiazem and simvastatin may undergo the same metabolic pathways and/or cellular transport mechanisms after co-administration. Therefore, simvastatin could affect the bioavailability or pharmacokinetics of diltiazem in rats. In the present study, cell-based P-gp activity tests using rhodamine-123 showed that simvastatin (10 μM) significantly ($p < 0.01$) inhibited P-gp and CYP3A4 activities (Figs. 1 and 2).

This result is consistent with previous reports. Bogman et al. [3] showed that simvastatin effectively inhibited P-gp activity; another study demonstrated that simvastatin effectively inhibited CYP3A4 activity [10]. Therefore, simvastatin, a dual inhibitor of both CYP3A4 and P-gp, may significantly impact the bioavailability of diltiazem, which is a substrate of CYP3A4 and P-gp. As CYP3A9 expressed in rat corresponds to the ortholog of CYP3A4 in human [19], and rat CYP3A2 is similar to human CYP3A4 [2]. Human CYP2C9 and 3A4 and rat CYP2C11 and 3A1 are 77% and 73% homologous, respectively [24].

As shown in Table 1, the presence of simvastatin significantly ($p < 0.05$) enhanced the AUC and C_{max} of diltiazem in rats. Subsequently, the RB of diltiazem was increased by 121–145% in the presence of sim-

vastatin (0.3 and 1.0 mg/kg). These results are consistent with previous reports that oral atorvastatin and fluvastatin significantly increased the bioavailability of diltiazem by inhibiting CYP3A and P-gp in rats [7, 17]. This suggests that the extraction ratio of diltiazem across rat intestinal tissue was significantly reduced by P-gp and/or CYP3A. These results were similar to those reported by Marumo et al. where pretreatment with simvastatin significantly enhanced the hypotensive effect of diltiazem, probably through competitive inhibition of diltiazem metabolism with simvastatin metabolism [25].

The MR in the presence of simvastatin was significantly ($p < 0.05$, 1.0 mg/kg) decreased compared to that of the control group. Lee et al. reported that the extraction ratios of diltiazem in the small intestine and liver after oral administration to rats were about 85% and 63%, respectively [21]. This suggests that diltiazem is highly extracted in both organs. Therefore, the decrease in intestinal extraction by the concomitant use of simvastatin resulted in enhanced diltiazem oral bioavailability.

In conclusion, the increased oral bioavailability of diltiazem in the presence of simvastatin might be due to inhibition of the P-gp-mediated efflux pump in the small intestine and CYP3A-mediated metabolism in the small intestine and/or liver by simvastatin, rather than renal elimination of diltiazem

Concomitant use of diltiazem with simvastatin may require close monitoring for potential drug interactions. However, the clinical importance of these findings should be further investigated in clinical trials.

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