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SHORT COMMUNICATION

Pharmacokinetic Interaction between Fluvastatin and Diltiazem in Rats

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ABSTRACT: The present study aimed to investigate the effect of fluvastatin on the pharmacokinetics of diltiazem in rats. Pharmacokinetic parameters of diltiazem were determined in rats following an oral administration of diltiazem (15 mg/kg) in the presence and absence of fluvastatin (0.6 and 2.0 mg/kg). Compared with the control given diltiazem alone, the C_{max} and *AUC* of diltiazem increased by 30–70% in rats with the concurrent use of fluvastatin, while there was no significant change in T_{max} and the plasma half-life ($T_{1/2}$) of diltiazem. Consequently, absolute and relative bioavailability values of diltiazem in the presence of fluvastatin were significantly higher ($p < 0.05$) than those from the control group, implying that fluvastatin could reduce the presystemic extraction of diltiazem. In conclusion, the concurrent use of fluvastatin significantly enhanced the oral exposure of diltiazem in rats. Copyright © 2006 John Wiley & Sons, Ltd.

Key words: diltiazem; pharmacokinetics; fluvastatin; drug interaction; rat

Introduction

Diltiazem is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension [1,2]. Since many patients with coronary artery disease also need lipid-lowering agents, diltiazem is often co-prescribed with lipid-lowering agents such as hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors [3,4]. Considering that diltiazem is known to be a substrate of CYP3A4, the pharmacokinetic and pharmacodynamic interactions may theoretically happen upon co-administration of diltiazem and HMG-CoA reductase inhibitors that are metabolized by CYP3A4. Indeed, previous pharmacokinetic studies have reported that calcium-channel blockers increased the plasma concentrations of simvastatin or lovastatin, probably by inhibiting CYP enzymes [5,6]. Kanathur *et al*. [7] have reported that the combined toxicities of rhabdomyolysis and hepatitis could be induced by the addition of diltiazem to simvastatin therapy. Given the prevalent co-morbidity of coronary artery disease, hypertension and hypercholesterolemia, analogous drug interaction may happen frequently and patients who require diltiazem therapy with the concurrent use of an HMG-CoA reductase inhibitor are therefore at risk for a drug interaction.

Fluvastatin was the first marketed synthetic HMG-CoA reductase inhibitor [8,9]. It is an antilipemic agent and also used as an adjunct to dietary therapy to slow the progression of coronary atherosclerosis in hypercholesterolemic patients suffering from coronary heart disease [8,9]. Fluvastatin is rapidly and completely absorbed from the gastrointestinal tract but

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undergoes extensive first-pass metabolism in the liver mainly by CYP 2C9 and CYP 3A4 [10–12]. CYP3A4 inhibitors (erythromycin, ketoconazole and itraconazole) have no effect on fluvastatin pharmacokinetics, in contrast to other HMG-CoA reductase inhibitors that are metabolized by CYP3A4 [13,14]. However, bi-directional pharmacokinetic interactions between fluvastatin and CYP3A4 substrates have not been evaluated and it is not clear yet whether fluvastatin can alter the pharmacokinetics of CYP3A4 substrates such as diltiazem.

Therefore, the present study aimed to investigate the effect of fluvastatin on the pharmacokinetics of diltiazem in rats.

Materials and Methods

Materials

Diltiazem hydrochloride, 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 of reagent grade and all solvents were of HPLC grade.

Animal studies

Male 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*. 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. Animals were kept in these facilities for at least 1 week before the experiment and fasted for 24 h prior to the experiments. In the previous study reported by Yeung *et al*. [15], the oral administration of diltiazem to rats at 15 mg/kg achieved a plasma level comparable to the therapeutic concentrations in humans. Therefore, in the present study, rats ($n = 6$ per each treatment) were given orally 15 mg/kg of diltiazem with either (i) fluvastatin (0.6 and 2.0 mg/kg), or (ii) no concomitant treatment (diltiazem alone). Separately, 5 mg/kg of diltiazem was administered intravenously to rats. Blood samples were collected from the femoral artery at 0, 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°C until analysed by HPLC.

HPLC assay

The plasma concentrations of diltiazem were determined by the HPLC assay modified from the method of Goebel *et al*. [16]. 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 0.01 N hydrochloride was added and mixed for 2 min. 50 µl of the water layer was 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), a 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 were as follows: diltiazem at 8.7 min and the internal standard at 9.7 min. The calibration curves of diltiazem were linear within the range of 10–1000 ng/ml. The intra-day ($n = 5$) and inter-day ($n = 5$) coefficients of variation were less than 5%. The detection limit of diltiazem was 10 ng/ml.

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

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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 $AUC_{oral}/AUC_{iv} \times Dose_{iv}/Dose_{oral} \times 100$, and the relative bioavailability (RB%) of diltiazem was estimated by $AUC_{diltiazem\ with\ fluvastatin}/AUC_{control} \times 100$.

Statistical analysis

All the means are reported with their standard deviation (mean ± SD). An unpaired Student's *t*-test was used to determine the significant difference between treatments. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

The mean plasma concentration-time profiles of diltiazem in the presence and absence of fluvastatin were characterized in rats and illustrated in Figure 1. The mean pharmacokinetic parameters of diltiazem were also summarized in Table 1. In the previous study reported by Yeung *et al*. [15], the oral administration of diltiazem to rats at 15 mg/kg achieved a C_{max} of 227.9 ± 320 ng/mL and *AUC* of 516 ± 560 ng/h/ml. In addition, Lee *et al*. [17] reported that the oral bioavailability of diltiazem was approximately 6% in rats. Therefore, the pharmacokinetic parameters of diltiazem obtained from the present study appeared to be comparable to those from the previous studies. However, the coadministration of fluvastatin (0.6 or 2.0 mg/kg) significantly altered the pharmacokinetic parameters of diltiazem compared with the control given diltiazem alone. The C_{max} and *AUC* of diltiazem increased by 30% to 70% in rats coadministered with fluvastatin, while there was no significant change in T_{max} or the terminal plasma half-life ($T_{1/2}$) of diltiazem in the presence of fluvastatin (Table 1). Consequently, the absolute and relative bioavailability values of diltiazem in rats coadministered

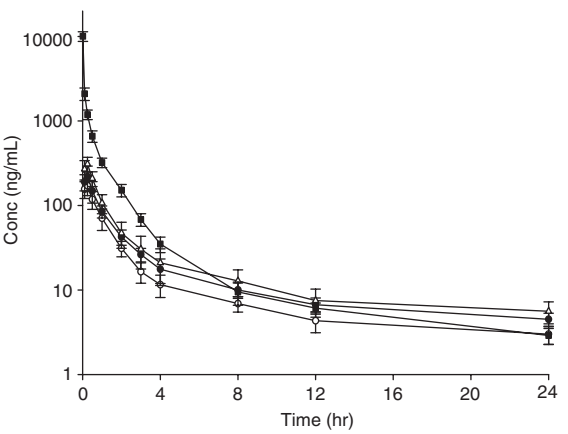

Figure 1. Mean plasma concentration-time profiles of diltiazem following intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of fluvastatin (mean ± SD, $n = 6$). (○) Control (diltiazem 15 mg/kg, oral), (●) coadministered with 0.6 mg/kg of fluvastatin, (△) coadministered with 2.0 mg/kg of fluvastatin, (■) i.v. injection of diltiazem (5 mg/kg)

Table 1. 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, $n = 6$)

Parameter	Diltiazem (control)	Diltiazem+Fluvastatin 0.6 mg/kg	2.0 mg/kg	i.v. 5 mg/kg
AUC (ng h/ml)	363 ± 63.9	477 ± 63.8[a]	628 ± 130[a]	1990 ± 178
C_{max} (ng/ml)	174 ± 35.8	223 ± 71.3	310 ± 62.1[a]	n.d.
T_{max} (h)	0.23 ± 0.06	0.23 ± 0.06	0.25	n.d.
$T_{1/2}$ (h)	12.1 ± 4.08	10.6 ± 2.60	11.8 ± 5.19	10.1 ± 1.69
AB (%)	6.1 ± 1.1	8.0 ± 1.1[a]	11 ± 2.2[a]	n.d.
RB (%)	100	131	180	n.d.

[a] $p < 0.05$, significant difference compared to the control (given diltiazem alone orally).
AB, absolute bioavailability, RB, relative bioavailability compared with the control group.
n.d., not determined.

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with fluvastatin were significantly higher ($p < 0.05$) than those from the control group.

Diltiazem is known as a substrate of P-gp and thus the intestinal absorption of diltiazem could be enhanced via the coadministration of P-gp substrates or inhibitors. However, Bogman *et al.* [18] reported that fluvastatin did not show any significant inhibition effect on P-gp while atorvastatin, lovastatin lactone and simvastatin lactone modulated the activity of P-gp. Therefore, drug interaction between fluvastatin and diltiazem via the inhibition of P-gp might be insignificant. As shown in Figure 1, while the slopes of the plasma concentration time curves were similar in all tested cases following the oral or intravenous administration of diltiazem, the concomitant use of fluvastatin increased the C_{max} and *AUC* of diltiazem. Those results suggest that fluvastatin could reduce the presystemic metabolism during intestinal absorption. Lee *et al.* [17] reported that the extraction ratios of diltiazem in the 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 both intestinal and hepatic extraction by the concomitant use of fluvastatin may contribute, at least in part, to the enhanced oral exposure of diltiazem.

Previous pharmacokinetic studies have reported that CYP3A4 inhibitors have no effect on fluvastatin pharmacokinetics, in contrast to other HMG-CoA reductase inhibitors such as simvastatin and lovastatin [13,14]. These results may be explained by the metabolic characteristics of fluvastatin. It has been reported that fluvastatin is mainly metabolized by CYP 2C9 (50–80%) and CYP 3A4 (~20%) with less contribution from CYP 2C8 (~5%) [11,12]. Therefore, fluvastatin can still undergo extensive metabolism by other CYP enzymes such as CYP2C9 even when the CYP3A4-mediated metabolic pathway is blocked in the presence of CYP3A4 inhibitors. Consequently, fluvastatin pharmacokinetics was not significantly altered by the concurrent use of CYP3A4 inhibitors. However, the results from the present study indicated that fluvastatin could be effective as an inhibitor to alter the pharmacokinetics of other CYP3A4 substrates such as diltiazem. Those results are also consistent with the previous report that fluvastatin inhibit the CYP 3A4-mediated metabolism of midazolam *in vitro* [19]. Therefore, in combination therapy, fluvastatin could affect the pharmacokinetics of co-prescribed drugs via the inhibition of CYP3A4-mediated metabolic pathway although the pharmacokinetics of fluvastatin itself may not be affected in the presence of CYP3A4 inhibitors. Since the present study raises awareness about the potential drug interaction by the concomitant use of fluvastatin with diltiazem, the clinical significance of this finding needs to be further evaluated in clinical studies.

In summary, the presence of fluvastatin significantly enhanced the oral exposure of diltiazem in rats, suggesting that the concurrent use of fluvastatin with diltiazem may require close monitoring for potential drug interactions.

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