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Effect of Oral Ketoconazole on Oral and Intravenous Pharmacokinetics of Simvastatin and Its Acid in Cynomolgus Monkeys

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Running title: Drug-drug interaction of simvastatin in monkeys

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Abbreviations: P450, cytochrome P450; SV, simvastatin; SVA, simvastatin acid, KTZ, ketoconazole; DDI, drug-drug interaction; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography tandem mass spectrometry; AUC, area under the plasma concentration-time curve; CL_{tot}, total body clearance; Vd_{ss}, distribution volume at steady state; C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}.

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Abstract

Drugs with potential drug-drug interactions (DDIs) may have a limited scope of use and, at worst, may have to be withdrawn from the market. Therefore, during the drug discovery process it is important to select drug candidates with reduced potential for DDIs. In the present study, we evaluated the pharmacokinetics of simvastatin (SV), a typical substrate for cytochrome P450 3A (P450 3A), and examined the DDI between SV and ketoconazole (KTZ), a P450 3A inhibitor, in monkeys. SV metabolism in monkey liver and intestinal microsomes was almost completely inhibited by addition of anti-P450 3A4 antiserum. A similar effect was seen in human microsomes and the IC_{50} values of KTZ for inhibition of SV metabolism were similar in monkey and human samples. *In vivo*, there were no significant differences in the pharmacokinetic parameters of SV and SVA after intravenous administration of SV in the presence of KTZ compared with those in controls, probably due to the limited systemic exposure to KTZ. In contrast, the pharmacokinetics of SV and SVA after oral administration of SV were significantly influenced by the presence of KTZ, and C_{max} and AUC were approximately five to ten times higher than those after oral dosing with SV alone. The increases in systemic SV exposure caused by a concomitant oral dose of KTZ in monkeys were similar to those observed in clinical studies, which suggests that monkeys might be a suitable animal model in which to predict DDIs involving P450 3A inhibition.

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Introduction

Drug-drug interactions (DDIs) caused by inhibition of drug-metabolizing enzymes can increase the risk of adverse events associated with drug administration, and some drugs with marked DDIs have had to be withdrawn from the market. Therefore, the potential for drug candidates to cause DDIs is usually assessed at an early stage in the drug discovery process. Many methods have been described for quantitative prediction of *in vivo* DDIs from *in vitro* data obtained using preparations of human tissues (Iwatsubo et al., 1997; Ito et al., 1998, 2004; Yamano et al., 2001; Obach et al.; 2006). These methods are widely accepted, but they tend to be used retrospectively and they are not applicable in all cases. In particular, knowledge of an inhibitor concentration around a drug-metabolizing enzyme is required to predict DDIs in humans accurately, but this concentration is difficult to estimate at an early stage of drug development.

There is increased interest in DDIs in the intestine and this has further complicated the prediction of DDIs. Several approaches have been proposed based on cytochrome P450 3A (P450 3A) inhibition in the intestine (Wang et al., 2004; Galetin et al., 2006, 2007; Obach et al., 2006), but predictions based on experimental data obtained in an *in vivo* animal model are limited because of substantial species differences in substrate specificities and inhibitor sensitivities of drug-metabolizing enzymes. Monkeys may be more appropriate animal models than rodents because of the similarities in functional activities and amino-acid sequences of drug-metabolizing enzymes compared to those in humans (Komori et al., 1992; Shimada et al., 1997), and it has been reported that human pharmacokinetics can be predicted more accurately from results obtained in monkeys compared to those from other animals, including rats and dogs (Ward et al., 2001; Ward

and Smith, 2004). In DDI studies, Tahara et al. (2006) have reported that human renal DDIs involving inhibition of drug transporters can be reproduced in monkeys, and we have evaluated the influence of ketoconazole (KTZ), a dual inhibitor of P450 3A and P-glycoprotein (P-gp), on the pharmacokinetics of midazolam and fexofenadine (as model P450 3A and P-gp substrates, respectively) in monkeys. Based on this work, we suggested that *in vivo* studies in monkeys might be appropriate for prediction of intestinal DDIs in humans, because our results were comparable to those in humans (Ogasawara et al., 2007). We conducted the current study of DDIs of simvastatin (SV) in a monkey model to examine the suitability of this animal species further.

SV is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor that is widely used for treatment of hypercholesterolemia. It is administered orally as a lactone prodrug, which is converted reversibly to active simvastatin acid (SVA) by nonspecific carboxyesterases in the intestinal wall and liver, and even without enzymes (Duggaw and Vickers, 1990; Vickers et al., 1990). The benefits of SV are well established, but occasionally it causes adverse events in skeletal muscle, including myopathy and rhabdomyolysis, and an increased risk of these adverse events has been reported with concomitant use of drugs such as KTZ, cyclosporine, fibrates, and erythromycin (Gilad and Lampl, 1999; Gruer et al., 1999; Williams and Feely, 2002). SV undergoes extensive first-pass metabolism mediated by P450 3A, resulting in extremely poor oral bioavailability (less than 5%) (Lilja et al., 1998), and the Food and Drug Administration (FDA) has suggested use of SV as a preferred *in vivo* probe with which to evaluate drug interactions related to P450 3A inhibition.

In the present study, we first determined the P450 isozyme involved in SV metabolism in

monkey liver and intestinal microsomes. We then investigated the pharmacokinetics of SV after intravenous and oral administration to monkeys to clarify the hepatic and intestinal contributions to the overall first pass metabolism. Finally, we evaluated the effects of KTZ on the pharmacokinetics of intravenously and orally administered SV to discriminate between hepatic and intestinal DDIs in monkeys.

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Materials and Methods

Chemicals. SV, SVA, SV d_6-form, and SVA d_6-form were purchased from Toronto Research Chemicals (North York, Canada). KTZ and polyethyleneglycol 400 were purchased from Wako Pure Chemical Industries (Osaka, Japan). Reserpine was obtained from Nacalai Tesque (Kyoto, Japan). Ibuprofen sodium salt and methyl cellulose (400 cps) were obtained from Sigma-Aldrich (St Louis, MO). Pooled liver and intestinal microsomes of human (liver microsomes; pooled from 50 donors, intestinal microsomes; pooled from 11 donors), monkey (liver microsomes; pooled from 5 animals, intestinal microsomes; pooled from 7 animals) and anti-human P450 antisera preparations (anti-P450 2C8, anti-P450 2C9, and anti-P450 3A4) were provided by Xenotech (Lenexa, KS). All other reagents and solvents were of analytical grade, and were commercially available.

Animals. Male cynomolgus monkeys, 3.0 to 4.5 kg, were supplied by the Yulin Hongfeng experimental animal's domesticating & breeding center (Guangxi, China) and Guangdong Zhaoquing Laboratory Animals Research Center Xiangang (Guangdong, China). Animals were housed in a temperature- and humidity-controlled room with a 12 hr light/dark cycle. Animals were fed a standard animal diet (Teklad Global Certified 25% Protein Primate Diet, Harlan Sprague Dawley, Indianapolis, IN); food was provided *ad libitum* except for during the overnight periods before dosing. Whenever overnight fasting was used, food was provided after 8 hr blood sample was obtained. All procedures for the animal experiments were approved by the Animal Ethics Committee of Tanabe Seiyaku.

***In vitro* inhibition studies.** Preliminary experiments were performed to determine

incubation conditions showing a linearity of the disappearance of SV with respect to the protein concentration and incubation time. Furthermore, it was confirmed that the elimination half-life of SV was almost constant at SV concentrations less than 0.5 μM. All incubations were performed in duplicate. A typical incubation contained 0.08 M potassium phosphate (pH7.4), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM NADPH, 0.5 μM SV, and microsomal protein in a final volume of 200 μL. The concentrations of microsomal protein in the reaction mixtures were as follows: human liver microsomes ; 0.06 mg/mL, human intestinal microsomes ; 0.2 mg/mL, monkey liver microsomes ; 0.02 mg/mL, monkey intestinal microsomes ; 0.06 mg/mL. After pre-incubation at 37ºC for 5 min, the reactions were initiated by the addition of the NADPH solution. After incubation at 37ºC for 3 min, aliquots of 50 μL were removed, and added to 50 μL of acetonitrile and methanol mixture (1 : 1, v/v). The samples were spiked with 20 μL of internal standard solution containing 50 ng/mL ibuprofen. After vortexing and centrifugation, the supernatants were transferred to an HPLC vial to determine the SV concentration by HPLC analysis.

The effect of P450 3A-selective inhibitor on SV metabolism was investigated using KTZ. Each microsomal protein was incubated with SV (0.5 μM) in the presence of KTZ at the concentrations of 0.01, 0.05, 0.25, and 1.25 μM. The final concentration of organic solvent in the incubation mixture was 1% (v/v).

In immuno-inhibition studies, human and monkey microsomes were pre-incubated at room temperature for 20 min with 50 μL/mg or 200 μL/mg microsomal protein of rabbit serum for human P450 2C8, 2C9, 3A4 or control serum. The SV metabolic reaction and subsequent treatment were carried out as described above.

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***In vivo* studies.** The oral and intravenous dosing solutions of SV were prepared in 0.5% (w/v) methylcellulose aqueous vehicle and saline containing 60% (v/v) polyethylenglycol 400 vehicle, respectively. The dosing solution of KTZ was prepared in ultra-pure water containing 0.01 mol/L hydrochloric acid. The same four male cynomolgus moneys were used again, after a washout period of at least two weeks, to investigate the effects of KTZ on the SV pharmacokinetics. Immediately after oral administration of KTZ (20 mg/kg), SV was administered orally or intravenously to the monkeys. The dosages of SV were 20 mg/kg and 1 mg/kg for oral and intravenous administration, respectively. To obtain the control values of the pharmacokinetic parameters for SV and SVA, the vehicle for KTZ was administered orally immediately before oral and intravenous administration of SV. For all studies, blood was collected from the femoral vein at the desired time points, and centrifuged at 1,800 g for 15 minutes at 4ºC to obtain plasma.

Analytical procedure. *Determination of SV concentrations in the microsomal reaction mixtures.* The SV concentrations in the microsomal reaction mixtures were measured using an Agilent 1100 system (Agilent Technologies, Palo Alto, CA). SV was eluted from a Capcell Pak C_{18} column (5 μm particle size, 4.6 × 150 mm, Shiseido, Tokyo, Japan) with a mobile phase of 0.025% (v/v) trifluoroacetic acid aqueous solution (pH 2.5) and acetonitrile. The flow rate was 1 mL/min, and the initial mobile phase was 50% of 0.025% trifluoroacetate aqueous solution and 50% of acetonitrile. The acetonitrile concentration was increased linearly to 90% over 8 min, and held at 90% for 2 min. The eluates were monitored at a wavelength of 240 nm. SV and the corresponding acid were eluted at 8.3 minutes and 6.9 minutes, respectively. The concentrations of SV were determined by its peak area ratio relative to an internal standard with reference to a

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standard curve using the ChemiStation version 10.02 software systems (Agilent).

Determination of SV and SVA concentrations in plasma samples. Twenty microliters of 60% (v/v) acetonitrile aqueous solution, 20 µL of internal standard solution containing 50 ng/mL of SV-d_6 and SVA-d_6, and 500 µL of 1 mmol/L ammonium acetate buffer solution were added to 100 µL aliquots of the plasma samples. The mixtures were loaded on Oasis HLB extraction cartridges (Waters, Milford, MA) which were pre-activated with 500 µL of methanol, water, and 1 mmol/L ammonium acetate buffer solution. The cartridges were subsequently washed twice with 500 µL of 5% (v/v) methanol aqueous solution, and then eluted twice with 100 µL of acetonitrile. One hundred microliters of the acetonitrile extracts were mixed with 100 µL of 1 mmol/L ammonium acetate buffer solution, and aliquots of the mixtures were analyzed by LC-MS/MS. Chromatography was performed using an Inertsil ODS-3 column (5 µm particle size, 2.1×50 mm, GL Science, Tokyo, Japan) and a HPLC system consisting of Shimazu 10A series (Shimazu, Kyoto, Japan) using a mobile phase consisting of 20% 1 mM ammonium acetate buffer solution and 80% acetonitrile. The mobile phase was delivered at a flow rate of 0.3 mL/min. Detection of the analyte and internal standard was performed using a Sciex API 4000 mass spectrometer (Applied Biosystems, Foster, CA) in the positive for SV and SV-d_6 and negative for SVA and SVA-d_6 ion mode using Turbo ion spray source at 300ºC. The collision gas was set at level 7. Mass transitions (m/z) monitored were 419 → 199 for SV, 435 → 319 for SVA, 425 → 199 for SV-d_6, and 441 → 319 for SVA-d_6 at collision energy of 17, -24, 17 and -24 eV, respectively. The lower limit of quantitification was 0.1 ng/mL for both SV and SVA. The standard curves were linear from 0.1 ng/mL to 200 ng/mL for both analytes. The between-day coefficient of

variation with CV and RE values for SV was 7.4% and -4.8% at 0.2 ng/mL (n=8), 2.9% and 1.8% at 10 ng/mL (n=8), and 3.5% and 0.9% at 160 ng/mL (n=8). The between-day coefficient of variation with CV and RE values for SVA was 11.7% and 3.1% at 0.2 ng/mL (n=8), 2.6% and 1.0% at 10 ng/mL (n=8), and 2.4% and 0.4% at 160 ng/mL (n=8).

Determination of KTZ concentrations in plasma samples. Ten microliters of acetonitrile and 10 µL of internal standard solution containing 1 µg/mL reserpine were added to 100 µL aliquots of the plasma samples. Aliquots of acetonitrile (200 µL) were added to the mixtures, and then they were stored at 4ºC for at least 1 hour. After centrifugation of 15,300×g at 4ºC for 5 min, 100 µL of the supernatants were added to the equal volume of 10 mmol/L formate ammonium solution, and aliquots of the mixtures were analyzed by LC-MS/MS. Chromatography was performed using an Unison UK-C8 column (3 µm particle size, 2.0×75 mm, Silvertone Science, Philadelphia, PA) and a HPLC system consisting of Shimazu 10A series (Shimazu, Kyoto, Japan). The mobile phase consisted of 10 mM formate ammonium solution and acetonitrile. The flow rate was 0.3 mL/min, and the initial mobile phase was 80% 10 mM formate ammonium solution and 20% acetonitrile. The acetonitrile concentration was increased linearly to 90% over 10 min. Detection of the analyte and internal standard was performed using a Sciex API 4000 mass spectrometer in the positive ion mode using Turbo ion spray source at 300ºC. The collision gas was set at level 7. Mass transitions (m/z) monitored were 531 → 489 for KTZ and 609 → 195 for internal standard, reserpine, respectively. The standard curves were linear from 0.1 ng/mL to 100 ng/mL for KTZ. The between-day coefficient of variation with CV and RE values for KTZ was 9.3% and -3.8% at 0.3 ng/mL (n=6), 3.4%

and -1.1% at 10 ng/mL (n=6), and 4.7% and -2.7% at 80 ng/mL (n=6).

Data Analysis. The percent activity remaining of SV metabolism was plotted against the range of KTZ concentrations on a semi-log scale. The IC_{50} values were determined by nonlinear regression analysis with Prism software package (version 3.02, GraphPad, San Diego, CA).

Pharmacokinetic parameters were calculated in individual animals by non-compartmental analysis using WinNonlin Professional (version 4.0.1, Pharsight, Mountain View, CA). The maximum plasma concentrations (C_{max}) and time to reach C_{max} (T_{max}) were directly recorded from experimental observations. The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule up to the last measurable concentrations. The bioavailability (F) was determined from the dose-corrected AUC following oral and intravenous administration. The components of SV availability were further predicted on the basis of the following equation:

$F = F_{ABS} \cdot F_G \cdot F_H$

in which F_{ABS} refers to the fraction of the dose absorbed from the gut lumen, F_G refers to the fraction of the dose not metabolized by intestinal metabolic enzymes, and F_H refers to the fraction of the dose absorbed into the hepatic vein that escapes first-pass effect in the liver. F_H was defined as follows:

$F_H = 1 - ER_H$

in which ER_H refers to the hepatic extraction ratio defined as the clearance of intravenously administered SV divided by the hepatic blood flow rate in monkeys (45 mL/min/kg, Davies and Morris, 1993) presuming that the non-hepatic contribution to the

clearance of SV was negligible, and that SV exhibited linear pharmacokinetics in the range of plasma concentrations observed in this study. The products $F_{ABS} \cdot F_G$ and F_H were determined, and compared with and without the presence of KTZ.

Statistical Analysis. All statistical tests were performed using the SAS v. 9.1.3 software (SAS Institute, Cary, NC). Statistical differences in the pharmacokinetic parameters were calculated by a two-tailed paired Student's *t*-test. In all cases, a probability level of $p < 0.05$ was considered significant.

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Results

Identification of P450 isoenzymes responsible for SV metabolism. To identify P450 isoform involved in SV metabolism, we evaluated the effects of KTZ, P450 3A specific inhibitor, on the metabolism of SV in human and monkey microsomes. SV metabolism in human liver and intestinal microsomes was inhibited by KTZ in a concentration-dependent manner, and the values of IC_{50} were estimated to be 0.023 μM and 0.051 μM, respectively (Table 1). As in human liver and intestinal microsomes, KTZ inhibited SV metabolism in monkey liver and intestinal microsomes with the IC_{50} values of 0.012 μM and 0.007 μM, respectively, which were similar to those in the respective human sample (Table 1). Furthermore, we evaluated the effects of anti-P450 antisera on SV metabolism to confirm the involvement of P450 3A isoform in SV metabolism. Anti-human P450 antisera was used in the immunoinhibition studies because it has been reported that these antibodies exhibit cross-reactivity with the corresponding monkey hepatic P450 isoforms (Shimada et al., 1997). In human and monkey microsomal samples, almost no effects were observed in SV metabolism in the presence of antibodies against human P450 2C8 and 2C9 even at the concentration of 200 μL/mg of microsomal protein (Figure 1). In contrast, SV metabolism in human and monkey microsomal samples was strongly inhibited by antibody against human P450 3A4. These results suggest that SV is metabolized primarily by P450 3A isozymes in humans and monkeys.

Pharmacokinetics of SV in cynomolgus monkeys. The mean plasma concentration-time profiles and pharmacokinetic parameters of SV and SVA after a single

intravenous administration of SV to the monkeys are presented in Fig. 2A and Table 2, respectively. The SV plasma concentrations declined rapidly in a monophasic manner with $T_{1/2}$ of 2.1 ± 0.8 hr. The CL_{tot} and Vd_{ss} were 1288 ± 110 mL/min/kg and 2567 ± 245 mL/kg, respectively. The maximum concentration of SVA (144.9 ± 10.2 ng/mL) was achieved at 0.083 hr, the first collection time, after intravenous dosing of SV (Fig. 3A, Table 3), and subsequently, SVA concentration declined in parallel with that of parent drug.

The plasma concentrations of SV reached C_{max} of 7.9 ± 6.1 ng/mL at 5.0 ± 2.6 hr after oral dosing of SV (Fig. 2B, Table 2). The oral bioavailability of SV was remarkably low (0.8 ± 0.6%). Following the oral dosing of SV, measurable amounts of SVA appeared in plasma at the first sampling time (0.25 hr, Fig. 3B, Table 3). Thereafter, the SVA concentration reached C_{max} of 2.9 ± 0.5 ng/mL at 1.5 ± 0.6 hr.

Effect of KTZ on the pharmacokinetics of SV and SVA in monkeys. To evaluate the effects of KTZ on the pharmacokinetics of SV and SVA, we determined SV and SVA concentrations in plasma after intravenous and oral administration of SV to the monkeys with concomitant oral dosing of KTZ. Fig.2 and Fig.3 depict the plasma concentration-time profiles of SV and SVA, respectively, in the presence and absence of KTZ. The effects of KTZ on the pharmacokinetic parameters of SV are summarized in Table 2. As shown in Fig. 2A and Table 2, the pharmacokinetic parameters of SV after intravenous dosing were not significantly affected with concomitant oral dose of 20 mg/kg KTZ. Furthermore, there were also no differences in the pharmacokinetic profiles of SVA with and without KTZ treatment (Fig. 3A, Table 3). In contrast, when

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SV was administered orally with concomitant oral dose of KTZ, the plasma concentrations of SV increased markedly (Fig. 2B, Table 2). The values of C_{max} and AUC for SV were increased 12.9 fold and 6.3 fold, respectively. Statistically significant increases were observed in the AUC and F for SV following oral coadministration with 20 mg/kg KTZ, compared with the control values. C_{max} for SV showed a tendency to increase, but this change was not statistically significant ($p = 0.0968$), probably due to the individual variability observed in monkeys with KTZ coadministration. Moreover, the plasma concentrations of SVA increased extremely as observed in the parent drug (Fig. 3B, Table 3). The values of C_{max} and AUC for SVA were increased 7.2 fold and 6.0 fold, respectively, and statistically significant increases were observed in these parameters.

Fig.4 and Table 4 represent the concentration-time profiles and pharmacokinetic parameters of KTZ, respectively, during both oral and intravenous administration of SV. There were no obvious differences between the pharmacokinetic parameters for KTZ with concomitant oral and intravenous dosing of simvastatin.

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Discussion

The risk of drug candidates causing pharmacokinetic DDIs is generally evaluated in *in vitro* studies of inhibition of drug-metabolizing enzymes at an early stage of drug development. However, the success of this approach varies because of the difficulty in estimating drug concentrations around drug-metabolizing enzymes, which is the most influential factor in predictive accuracy. It has been proposed that prediction of DDIs in *in vivo* animal models is necessary for more accurate results (Ohba et al., 1996; Tsuruta et al., 1997; Ward et al., 2001), but a number of studies have demonstrated species-related differences in the catalytic roles of P450 isoforms for drugs in animals and humans. In the present study, we determined the P450 isozymes involving in SV metabolism in monkeys using anti-P450 antisera and KTZ, a potent P450 3A inhibitor. SV metabolism in monkey liver and intestinal microsomes was almost completely inhibited by addition of anti-P450 3A4 antiserum, and a similar effect was observed in human microsomes. In contrast, anti-P450 2C8 or 2C9 antiserum had no inhibitory effect in either human or monkey microsomes. The IC_{50} values of KTZ in monkey liver and intestinal microsomes were extremely low, as also observed in human microsomes.

Based on the *in vitro* results, we conclude that the P450 3A isozyme expressed in monkey liver and intestine is predominantly involved in SV metabolism. In a study of inhibition of midazolam metabolism, Kanazu et al. (2004) showed that the apparent K_i values and inhibitory mechanisms of KTZ, erythromycin and diltiazem, all of which are typical P450 3A inhibitors, were similar in human and monkey liver microsomes, and we have demonstrated the similarity of inhibitor sensitivities for midazolam hydroxylation in human and monkey intestinal and liver microsomes (Ogasawara et al., 2007). Taken

together, these results suggest that a monkey model may be useful for assessment of human DDIs associated with P450 3A inhibition in the liver and intestine. P450 3A8, which exhibits more than 90% amino-acid sequence identity with P450 3A4, is the only reported P450 3A isoform in cynomolgus monkeys (Komori et al., 1992) and represents about 20% of the P450 content in monkey liver, comparable with the proportion of P450 3A4 in human liver. In monkey intestine, P450 MI-1, MI-2 and MI-3 have been identified as P450 isoforms associated with ebastin metabolism (Hashizume et al., 2001). It has been suggested that P450 MI-3 belongs to the P450 3A subfamily based on the N-terminal amino-acid sequence, immunoblot analysis, and inhibition studies using antibodies and KTZ.

It has been widely believed that the liver is the major site of first-pass metabolism of orally administered drugs, but recent studies have indicated that the intestine also makes a significant contribution to the overall first-pass metabolism. In fact, a number of drugs that are solely metabolized by P450 3A undergo significant first-pass metabolism in the intestine (Gomea et al., 1994; Floren et al., 1996; Thummel et al., 1996; Masica et al., 2004). In the present study, we evaluated SV pharmacokinetics in monkeys to estimate the relative significance of the liver and intestine in first-pass metabolism of SV. Our results indicate that monkey intestine is a predominant site for first-pass metabolism of SV because the intestinal availability (F_G) was much lower than the hepatic availability (F_H) (1.6% vs. 52%, Table 2), assuming that the oral dose of SV was almost completely absorbed into the gut wall regardless of species (Vickers et al., 1990) and that the non-hepatic contribution to the clearance of SV was negligible. Regarding the intestinal contribution to first-pass metabolism of SV in humans, Obach et al. (2006) have

estimated F_H and F_G to be 0.67 and 0.66, respectively. Therefore, our results suggest that F_H values in monkeys are comparable to those in humans, but that there is a significant difference in the intestinal contribution to the overall first-pass metabolism in monkeys and humans. However, Lilja et al. (1998) reported that concomitant administration of SV with grapefruit juice, which influences intestinal P450 3A expression with no effect on hepatic P450 3A expression and activity, increased the AUC of SV by 16-fold in humans. The increase in the AUC of SV with grapefruit juice suggests that F_G for SV may be extremely low compared with F_H in humans, as in monkeys. Further investigations are required to clarify the relative contribution of the liver and intestine to the overall first-pass metabolism of SV in humans.

The risk of myopathy with SV alone is dose related and this risk is increased by concomitant use of the P450 3A inhibitors such as KTZ, cyclosporine, fibrates and erythromycin (Gilad and Lampl, 1999; Gruer et al., 1999; Williams and Feely, 2002). In the present study, KTZ had a significant influence on the pharmacokinetic profiles of SV and SVA following oral administration of SV to monkeys, with C_{max} and AUC of SV increasing by 12.9-fold and 6.3-fold, respectively, in the presence of KTZ (Fig. 2, Table 2). Moreover, co-administration of KTZ led to a significant increase in C_{max} and AUC of SVA, the main active metabolite of SV (Fig. 3, Table 3). In contrast, there were no significant changes in the pharmacokinetic parameters of SV and SVA following intravenous SV administration in the presence of KTZ. These results indicate that KTZ does not inhibit hepatic SV metabolism in monkeys, suggesting that the KTZ-mediated increase in SV bioavailability may be attributable predominantly to decreased intestinal metabolism, resulting in the increased F_G. A clinical study showed that C_{max} and AUC of

SV after oral administration to humans increased by 7.4-fold and 12.6-fold, respectively, after pretreatment with 400 mg KTZ once a day for 10 days (Chung et al., 2006). Furthermore, unlike in monkeys, KTZ also influenced hepatic SV metabolism, resulting in prolongation of the terminal half-life of oral SV by 1.9-fold in the presence of KTZ. The KTZ plasma concentration in humans reaches 10.2 μg/mL after oral administration of 400 mg KTZ once a day for 10 days (Khaliq et al., 2000), whereas the plasma concentration of KTZ in monkeys after 20 mg/kg oral dosing was about one-sixteenth of that in humans. Therefore, the apparent species differences in the effect of KTZ on hepatic SV metabolism may be due to a difference in the systemic exposure level to KTZ in monkeys and humans. A further investigation in which KTZ is dosed intravenously prior to SV administration may be necessary to clarify the effect of KTZ on hepatic SV metabolism in monkeys. Regarding DDIs associated with SV in humans, there are no data that permit discrimination of the influence of KTZ on F_G and F_H of oral SV. However, considering the significant SV metabolism in the intestine and the susceptibility of SV metabolism to KTZ, it is possible that the increased C_{max} and AUC of SV can be attributed predominantly to decreased intestinal metabolism, as observed for other drugs metabolized by P450 3A4 (Gomes et al., 1994; Floren et al., 1996).

Several studies have evaluated the applicability of animal models for prediction of human DDIs. Ishigami et al. (2001) examined whether the interaction between SV and itraconazole observed in humans also occurs in rats. The AUC of SV was slightly increased (approximately 1.6-fold) in female rats with concomitant use of itraconazole, even at a dose of 50 mg/kg, but this increase was significantly less than that observed in a clinical situation (more than 10-fold, Neuvonen et al., 1998). It was concluded that this

might be due to species differences in the metabolic pathways and P450 isoforms involved in SV metabolism. Similarly, Kotegawa et al. (2002) evaluated the DDI between midazolam and KTZ in rats and concluded that the clinical applicability of the rat model is limited by species differences in patterns of metabolic biotransformation and systemic pharmacokinetics, as well as susceptibility to inhibition by chemical inhibitors. In contrast, the results presented herein indicate that the DDI between SV and KTZ in humans is reproduced in monkeys, as observed in our previous work using midazolam and KTZ (Ogasawara et al., 2007).

In conclusion, we have demonstrated that the oral bioavailability of SV is extremely low in monkeys and that intestinal metabolism contributes extensively to the overall availability. When SV was administered intravenously, the plasma concentration-time profiles of SV and SVA were not influenced by oral coadministration of KTZ because of the limited systemic exposure of KTZ. In contrast, SV oral bioavailability was increased significantly by a concomitant oral dose of KTZ, probably due to inhibition of SV intestinal metabolism. Since these results are comparable to those in humans, we suggest that monkeys might be a suitable animal model in which to predict DDIs involving P450 3A inhibition. Further studies using other P450 3A substrates and inhibitors are needed to confirm the suitability of this model.

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Legends for Figures

Figure 1 Effects of anti-human P450 antisera on SV metabolism in human and monkey microsomes.

Before determining SV metabolic activity, human and monkey microsomes were pre-incubated at room temperature for 20 min with 50 μL/mg or 200 μL/mg microsomal protein of rabbit serum for human P450 2C8, 2C9, 3A4 or control serum. Bars represent the mean of duplicate measurements.

Figure 2 Effects of oral coadministration of KTZ (●; 0 mg/kg, ○; 20 mg/kg) on the plasma concentration-time profiles of SV after intravenous (A) and oral (B) administration to monkeys at the doses of 1 and 20 mg/kg, respectively.

Each point represents the mean ± S.D. of data obtained from four monkeys, except where denoted. The points with an asterisk indicate the mean ± S.D. of three monkeys or the mean of two monkeys.

Figure 3 Effects of oral coadministration of KTZ (●; 0 mg/kg, ○; 20 mg/kg) on the plasma concentration-time profiles of SVA after intravenous (A) and oral (B) administration of SV to monkeys at the doses of 1 and 20 mg/kg, respectively.

Each point represents the mean ± S.D. of data obtained from four monkeys, except where denoted. The points with an asterisk indicate the mean ± S.D. of three monkeys or the mean of two monkeys.

Figure 4 Plasma concentration-time profiles of KTZ after oral administration to

monkeys concomitant with intravenous (A) and oral (B) dosing of SV.

Each point represents the mean ± S.D. of data obtained from four monkeys.

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Table 1. IC_{50} values of KTZ for SV metabolism in human and monkey microsomes.

SV (0.5μM) was incubated at 37ºC for 3 minutes with human and monkey microsomes in the absence or presence of various concentrations of KTZ.

Microsomes		IC_{50} (μM)
Human	Liver	0.023
	Intestine	0.051
Monkey	Liver	0.012
	Intestine	0.007

Each value represents the mean of duplicate measurements.

Table 2. Pharmacokinetic parameters of SV following intravenous (1 mg/kg) or oral (20 mg/kg) administration of SV with a concomitant oral dose of vehicle or KTZ (20 mg/kg).

		Intravenous (1 mg/kg, SV)		Oral (20 mg/kg, SV)	
		+ Vehicle	+ KTZ (20 mg/kg)	+ Vehicle	+ KTZ (20 mg/kg)
C_{max}	(ng/mL)	-	-	7.9 ± 6.1	104.9 ± 86.3
T_{max}	(hr)	-	-	5.0 ± 2.6	4.0 ± 2.3
$T_{1/2}$	(hr)	2.1 ± 0.8	1.9 ± 0.6	-	4.8 ± 2.9
CL_{tot}	(mL/hr/kg)	1288 ± 110	1309 ± 110	-	-
Vd_{ss}	(mL/kg)	2567 ± 245	2770 ± 507	-	-
AUC	(hr•ng/mL)	773 ± 64	762 ± 68	120 ± 95	587 ± 343*
F	(%)			0.8 ± 0.6	3.8 ± 2.1*
F_H	(%)	52 ± 4	52 ± 4		
F_{ABS}•F_G	(%)			1.6 ± 1.3	7.5 ± 4.2
Ratios (KTZ/Vehicle)					
C_{max}			-		12.9 ± 4.6
AUC			1 ± 0.1		6.3 ± 2.6
F			-		6.3 ± 2.6
F_H			1 ± 0.1		-
F_{ABS}•F_G			-		6.6 ± 2.8

Each value represents the mean ± S.D. of four monkeys.

* $p < 0.05$ compared with the values in the monkeys coadministered with vehicle for KTZ.

Table 3. Pharmacokinetic parameters of SVA following intravenous (1 mg/kg) or oral (20 mg/kg) administration of SV with a concomitant oral dose of vehicle or KTZ (20 mg/kg).

		Intravenous (1 mg/kg, SV)		Oral (20 mg/kg, SV)	
		+ Vehicle	+ KTZ (20 mg/kg)	+ Vehicle	+ KTZ (20 mg/kg)
C_{max}	(ng/mL)	144.9 ± 10.2	128.9 ± 11.4	2.9 ± 0.5	20.9 ± 8.1*
T_{max}	(hr)	0.083 ± 0.000	0.125 ± 0.084	1.5 ± 0.6	4.0 ± 2.3
$T_{1/2}$	(hr)	1.4 ± 0.2	1.5 ± 0.2	-	6.3 ± 7.0
AUC	(hr•ng/mL)	284 ± 24	288 ± 19	26 ± 14	130 ± 31**
Ratios (KTZ/Vehicle)					
C_{max}			0.9 ± 0.1		7.2 ± 2.5
AUC			1.0 ± 0.1		6.0 ± 3.0

Each value represents the mean ± S.D. of four monkeys.

$p < 0.05$ (*) , $p < 0.01$ (**) compared with the values in the monkeys coadministered with vehicle for KTZ.

Table 4. Pharmacokinetic parameters of KTZ following oral (20 mg/kg) administration of KTZ with intravenous or oral co-administration of SV.

		Oral (20 mg/kg, KTZ)	
		+ SV (i.v., 1 mg/kg)	+ SV (p.o., 20 mg/kg)
C_{max}	(ng/mL)	719 ± 637	621 ± 569
T_{max}	(hr)	2.5 ± 1.0	3.5 ± 1.9
$T_{1/2}$	(hr)	4.6 ± 0.7	3.0 ± 0.6
AUC	(hr•ng/mL)	1710 ± 1325	2167 ± 814

Each value represents the mean ± S.D. of four monkeys.

Figure 1

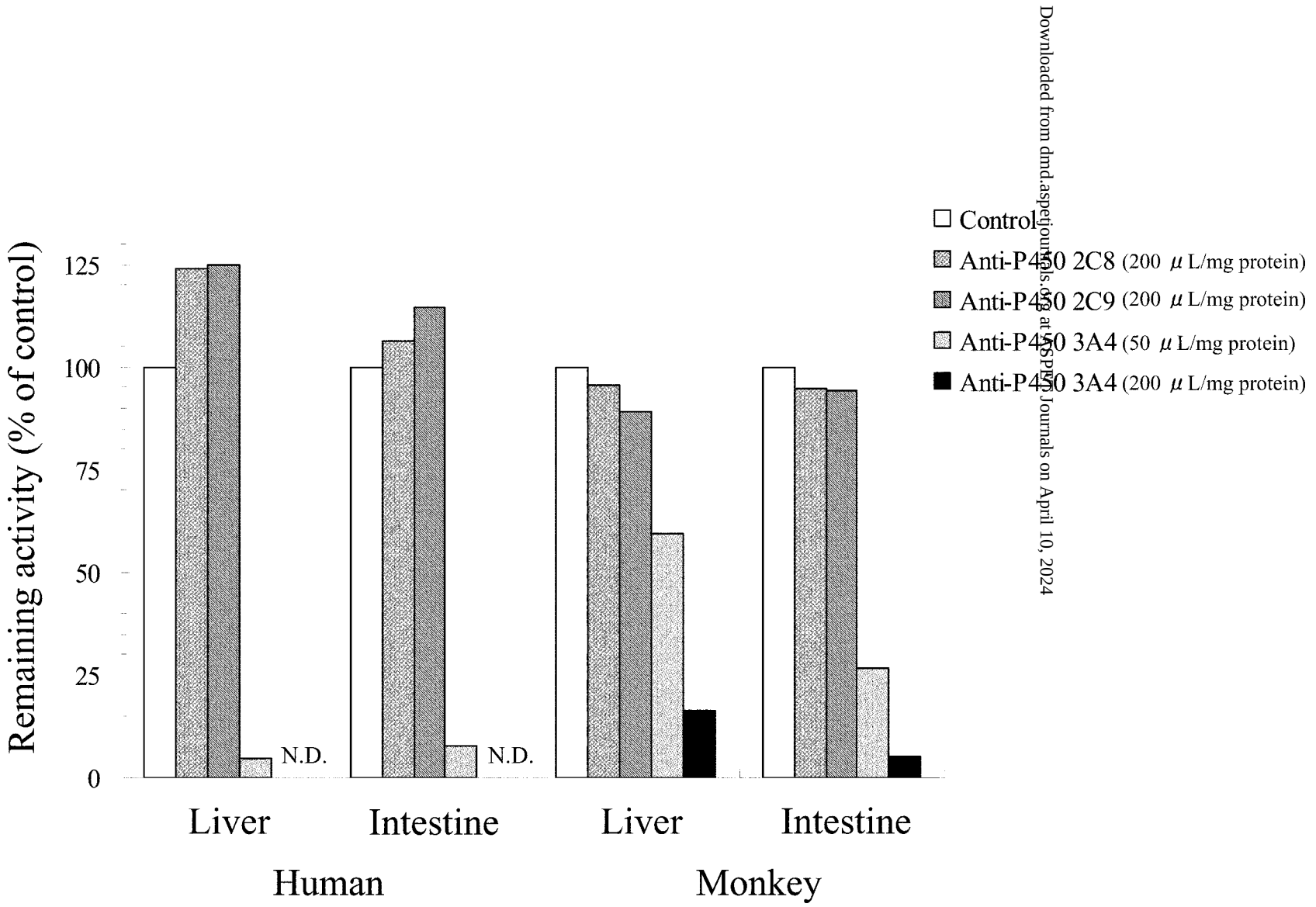

Figure 2

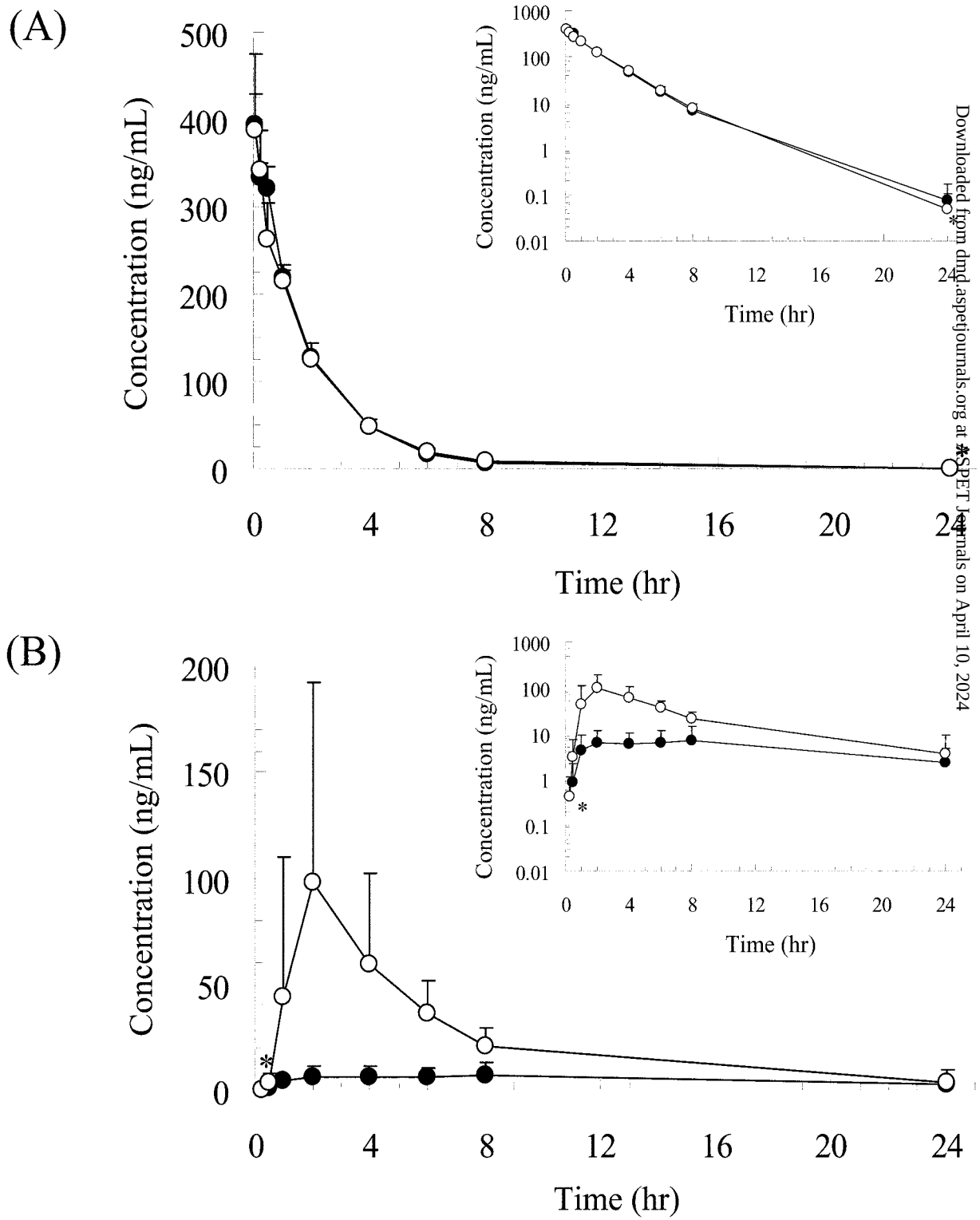

Figure 3

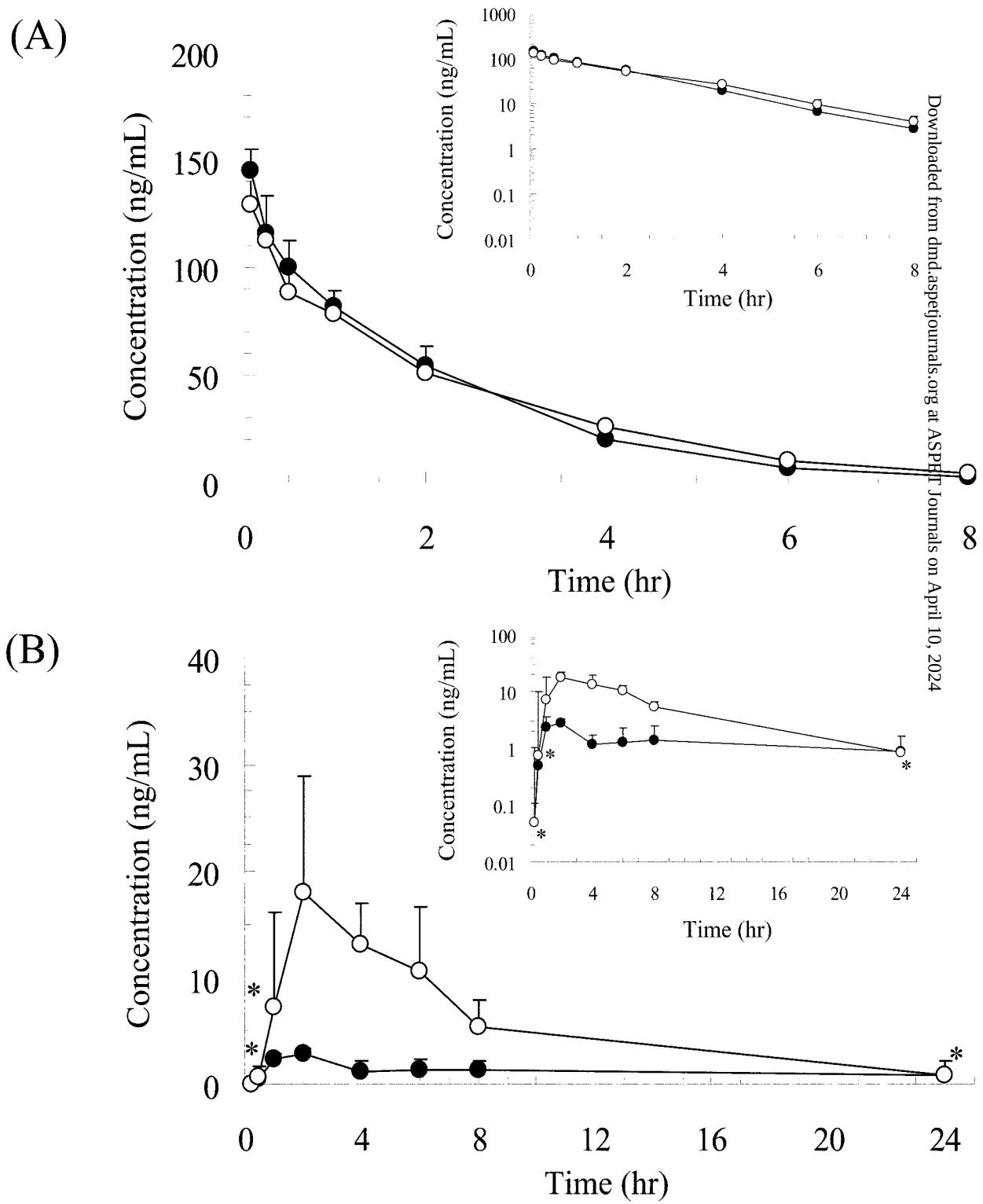

Figure 4