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**Investigation of the Rate-determining Process in the Hepatic Elimination of HMG-CoA
Reductase Inhibitors in Rats and Humans**

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Abbreviations

OATP, organic anion transporting polypeptide; statin, HMG-CoA reductase inhibitor; AUC, area under the concentration–time curve; PS, permeability surface area product; CL, clearance; $CL_{int,all}$, overall intrinsic clearance; inf, influx; eff, efflux; met, metabolism; tot, total; B, blood;

FD-4, fluorescein isothiocyanate dextran 4000; MID, multiple indicator dilution; R_B , blood-to-plasma concentration ratio; f_B , unbound fraction in blood; F_H , hepatic availability; BSA, bovine serum albumin; CYP, cytochrome P450

Abstract

Elucidation of the rate-determining process in the overall hepatic elimination of drugs is critical for predicting their intrinsic hepatic clearance, and the impact of variation of sequestration clearance on their systemic concentration. The present study investigated the rate-determining process in the overall hepatic elimination of the HMG-CoA reductase inhibitors, pravastatin, pitavastatin, atorvastatin, and fluvastatin, both in rats and humans. The uptake of these statins was saturable in both rat and human hepatocytes. Intrinsic hepatic clearance obtained by *in vivo* pharmacokinetic analysis in rats was close to the uptake clearance determined by the multiple indicator dilution method but much greater than the intrinsic metabolic clearance extrapolated from an *in vitro* model using liver microsomes. *In vivo* uptake clearance of the statins in humans (pravastatin 1.44, pitavastatin 30.6, atorvastatin 12.7, and fluvastatin 62.9 ml/min/g liver), which was obtained by multiplying *in vitro* uptake clearance determined in cryopreserved human hepatocytes by rat scaling factors, were within the range of overall *in vivo* intrinsic hepatic clearance (pravastatin 0.84–1.2, pitavastatin 14–35, atorvastatin 11–19, and fluvastatin 123–185 ml/min/g liver), whereas the intrinsic metabolic clearance of atorvastatin and fluvastatin were considerably low compared with their intrinsic hepatic clearance. Their uptake is the rate-determining process in the overall hepatic elimination of the statins in rats, and this likely holds true in humans. *In vitro*–*in vivo* extrapolation of the uptake clearance using a cryopreserved human hepatocytes model and rat scaling factors will be effective for predicting *in vivo* intrinsic hepatic clearance involving active uptake.

Introduction

Predicting the pharmacokinetic properties of drug candidates in preclinical stages of development has been a critical issue for avoiding failure in clinical stages of development because of pharmacokinetics. The liver is the major clearance organ for drugs in the body where the inactivation mechanisms are comprised of metabolic enzymes and drug transporters. These inactivation mechanisms are associated with the hepatic first-pass effect following oral administration as well as with elimination from the systemic circulation. It is well accepted that, because of large species differences in drug metabolism, the results of animal studies cannot be directly extrapolated to humans. Instead, *in vitro* systems have been developed to replace animal studies and provide reliable predictions. In particular, human liver microsomes enable the reliable prediction of the metabolic clearance of drugs in the liver of humans (Iwatsubo et al., 1997; Obach, 1999; Naritomi et al., 2001; Kilford et al., 2008; Stringer et al., 2008). It has been found that the substrates of hepatic uptake transporters, organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3, include anionic drugs whose major elimination pathway is metabolism by cytochrome P450 (CYP) and UDP-glucuronosyltransferase in the liver. These drugs include cerivastatin, atorvastatin, fluvastatin, repaglinide, and telmisartan (Fischer et al., 1999; Jacobsen et al., 2000; Bidstrup et al., 2003; Kirchheiner et al., 2003; Shitara and Sugiyama, 2006). These drugs have been considered as outliers in the prediction of elimination using human liver microsomes because of active transport in their uptake process, concentrating substrate drugs inside the cells.

For these drugs, the impact of variation of sequestration clearance on the drug concentrations in the systemic circulation depends on a rate-limiting process (Kusuhara and Sugiyama, 2009). Despite its importance for predicting the impact of variation of metabolic activity or canalicular efflux on systemic exposure, there are only a few studies examining the rate-determining process for the hepatic elimination of pravastatin and methotrexate in rats

(Yamazaki et al., 1996; Ueda et al., 2001). Because of the lack of information regarding liver concentrations of these drugs, the rate-determining process in their overall hepatic elimination has not been investigated in humans. We proposed an in vitro–in vivo extrapolation (IVIVE) in which the uptake clearance is extrapolated from an in vitro model using cryopreserved human hepatocytes and rat scaling factor based on the finding that scaling factors for CYP-mediated metabolism are preserved across the species (Naritomi et al., 2001). The extrapolated uptake clearance of pravastatin was within the range of clinically reported intrinsic hepatic clearance, suggesting that the uptake is also the rate-determining process in humans (Watanabe et al., 2009). The purpose of this study was to apply this method to the other HMG-CoA reductase inhibitors (statins), pitavastatin, atorvastatin, and fluvastatin, in rats and humans. Hepatic elimination is the major pathway for elimination of these statins from the systemic circulation, the mechanisms involved are different; atorvastatin and fluvastatin are metabolized by CYP3A4 and 2C9, respectively, and pitavastatin undergoes biliary excretion by BCRP (Fischer et al., 1999; Jacobsen et al., 2000; Kirchheiner et al., 2003; Hirano et al., 2005). Hepatic uptake of pravastatin, pitavastatin and atorvastatin involves a transporter, OATP1B1, based on a kinetic analysis of pitavastatin using human hepatocytes (Hirano et al., 2004), and clinical studies for pravastatin, pitavastatin and atorvastatin. Generic variation of OATP1B1, such as OATP1B1*5 and *15, shows reduced transport activities compared with the reference OATP1B1 (OATP1B1*1a) (Tirona et al., 2001; Iwai et al., 2004; Nozawa et al., 2005), and healthy volunteers carrying those genotypes exhibits greater systemic exposure of pravastatin, pitavastatin and atorvastatin, indicating the importance of OATP1B1 in their hepatic uptake process (Nishizato et al., 2003; Maeda et al., 2006; Niemi et al., 2006; Ieiri et al., 2007; He et al., 2009). On the other hand, the systemic exposure of fluvastatin was independent of OATP1B1 genotype (Niemi et al., 2006), whereas fluvastatin is a substrate of OATP1B1 (Kopplow et al., 2005; Noe et al., 2007). OATP1B1 is suggested to only make a negligible contribution to the hepatic elimination of fluvastatin.

In the present study, the overall intrinsic hepatic clearances of the statins were determined from in vivo studies using rats, and their uptake clearances were determined using a multiple indicator dilution (MID) technique. Metabolic clearances were determined using liver microsomes. In addition, in vitro parameters for hepatic uptake and metabolism were determined using cryopreserved human hepatocytes and liver microsomes, and extrapolated to the corresponding in vivo parameters to compare these parameters with clinically determined intrinsic hepatic clearances. The present study suggests that hepatic uptake is the predominant factor for hepatic elimination of these representative statins.

Methods

Materials

Pravastatin and a pravastatin analogue, R-122798 ((3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8*aR*)-6-hydroxy-8-(isobutyryloxy)-2-methyl-1,2,6,7,8,8*a*-hexahydronaphthalen-1-yl]heptanoic acid), were kindly donated by Daiichi Sankyo Co. (Tokyo, Japan). Pitavastatin was kindly donated by Kowa Co. (Tokyo, Japan). Atorvastatin was purchased from AK Scientific (Mountain View, CA). Fluvastatin and cerivastatin were purchased from Toronto Research Chemicals (North York, ON, Canada). Fluorescein isothiocyanate dextran 4000 (FD-4, 4000 Da) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of analytical grade and were readily available from commercial sources.

Animals

Male Sprague Dawley (SD) rats (6–7 weeks old) were purchased from Nippon SLC (Shizuoka, Japan). All animals were maintained under standard conditions with a reversed light/dark cycle and were treated humanely. Food and water were available ad libitum. The studies were conducted in accordance with the guidelines of the Institutional Animal Care Committee, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.

Preparation of rat and human hepatocytes

Isolated rat hepatocytes were prepared from SD rats by the collagenase perfusion method described previously (Yamazaki et al., 1993). Isolated hepatocytes (viability > 88%) were suspended in Krebs–Henseleit buffer, adjusted to 2.0×10^6 cells/ml, and stored on ice before the uptake experiment. Cryopreserved human hepatocytes were purchased from Xenotech LLC (Lenexa, KS), the Research Institute for Liver Disease (Shanghai, China) and In Vitro Technologies (Baltimore, MD). Just before the uptake experiment, the hepatocyte suspension

was thawed at 37 °C and poured into Tube A of the hepatocyte isolation kit (Xenotech, LLC) containing supplemented DMEM and isotonic Percoll and then centrifuged ($70 \times g$) for 5 min at 25 °C. After the supernatant was removed, the cells were resuspended in 5 ml of supplemented DMEM in Tube B of the hepatocyte isolation kit. The number of viable cells was then determined using trypan blue staining. The cell viability of human hepatocytes ranged from 75% to 97%. Subsequently, the cells were resuspended in the remaining medium from Tube B (approximately 40 mL) and then centrifuged ($50 \times g$) for 3 min at 25 °C, followed by removal of the supernatant. Finally, the cells were resuspended in the Krebs–Henseleit buffer at a density of 2.0×10^6 viable cells/ml for the uptake experiment.

Determination of statin uptake clearance using hepatocytes

This experiment was performed as described previously with a minor modification (Hirano et al., 2004). Before the uptake studies, the cell suspensions were prewarmed at 37 °C for 3 min. The uptake reaction was initiated by adding an equal volume of buffer containing drugs to the hepatocyte suspension. After incubation at 37 °C for 0.5, 1.5, and 2.5 min, the reaction was terminated by separating the cells from the substrate solution. For this purpose, an aliquot of 80–100 μ l of incubation mixture was placed in a 0.4 ml centrifuge tube containing 50 μ l of 5 M ammonium acetate under a 100 μ l layer of oil mixture (density, 1.015, a mixture of silicone oil and mineral oil; Sigma-Aldrich), and subsequently the sample tubes were centrifuged for 15 s using a tabletop centrifuge (10,000g, MC-150, TOMY SEIKO Co., Tokyo, Japan). During this process, hepatocytes passed through the oil layer into the aqueous solution. Tubes were frozen in liquid nitrogen immediately after centrifugation and stored at –30 °C until quantification. An aliquot was taken from the upper media portion and quenched in methanol, and the cells were taken from the centrifuge tube and sonicated in a new tube, containing methanol, to disintegrate them. The samples were vortexed and centrifuged, and supernatants from both the media and cell portions were analyzed by liquid chromatography–tandem mass

spectrometry (LC–MS/MS). The area under the statin concentrations in the incubation buffer (AUC_{buf}^{0-t}) was calculated using a trapezoidal method. The amount of statin uptake into hepatocytes per 10^6 viable cells (X_{hep}) normalized by the buffer concentration (C_{buf}) can be described by the following equation:

$$\frac{X_{hep}}{C_{buf}} = PS_{inf,vitro} \times \frac{AUC_{buf}^{0-t}}{C_{buf}} + V_0 \quad (1)$$

where $PS_{inf,vitro}$ and V_0 represent uptake clearance into hepatocytes and the initial distribution volume, respectively. Based on Eq. 1, the $X_{hep}(t)/C_{buf}(t)$ value was plotted against the $AUC_{buf}^{0-t}/C_{buf}(t)$ value, and $PS_{inf,vitro}$ was determined as the initial slope of the plot and expressed as the in vitro uptake clearance ($\mu\text{L}/\text{min}$ per 10^6 cells). A physiological scaling factor of 1.2×10^8 cells/g liver was used for scaling up to the organ level (Iwatsubo et al., 1997).

In vivo pharmacokinetic analysis in rats

Male SD rats, weighing approximately 240–300 g, were used for these experiments. Under ether anesthesia, the femoral artery was cannulated with a polyethylene catheter (SP-31) for the collection of blood samples. The bile duct was cannulated with a polyethylene catheter (PE-10) for bile collection, and the bladder was cannulated with a silicon catheter to collect urine. The femoral vein was cannulated with a polyethylene catheter (SP-31) for the administration of statins. Each rat was placed in a Bollman cage and allowed to recover from the anesthesia before the experiments were continued. The rats were given statins intravenously at 1 $\mu\text{mol}/\text{kg}$ (pitavastatin and atorvastatin) or 0.5 $\mu\text{mol}/\text{kg}$ (fluvastatin). Blood samples were collected at the designated times and centrifuged at 1500g for 10 min at 4 °C to obtain plasma. Bile and urine samples were collected in preweighed test tubes at the designated intervals throughout the experiment. All the samples were stored at –30 °C until quantification. Plasma, bile, and urine samples were deproteinized with two volumes of methanol and centrifuged at 15,000g for 10 min at 4 °C. The supernatant was subjected to LC–MS/MS analysis.

Liver perfusion study (Multiple Indicator Dilution method)

The procedures are basically as reported (Miyauchi et al., 1993; Akita et al., 2002). Under ether anesthesia, the portal and hepatic veins were cannulated to allow infusion of the perfusate and to allow the outflow to be collected, respectively. The perfusate consisted of 3% bovine serum albumin (BSA) in the Krebs–Ringer bicarbonate buffer (pH 7.4), and the flow rate was 30 ml/min. After the stabilization period of 10 min, 200 µl of the perfusion solution containing FD-4 (100 µM), an extracellular reference, and each statin (50 µM) was administered as a bolus into the portal vein. After administration, the total effluent from the hepatic venous vein was collected at 1 s intervals for 10 s. The concentration of FD-4 and statins in the collected samples were determined using a fluorescence plate reader (FluoStar Optima, BMG Labtechnologies, Offenburg, Germany; 485 nm for excitation and 520 nm for emission) and by LC–MS/MS, respectively. The natural logarithm of the ratio of FD-4 to statin concentration in the outflow was plotted as a function of time. The initial slope of this plot, calculated by linear regression analysis using initial four to five data points, reflects the influx rate constant (K_1). The unbound uptake clearance ($PS_{inf,MID}$) can be calculated by the following equation (Eq. 2):

$$f_u \cdot PS_{inf,MID} = K_1 \times V_{ext} \quad (2)$$

where f_u and V_{ext} represent the unbound fraction of statins in the perfusion buffer containing BSA and the extracellular volume, which can be estimated by multiplying the perfusate flow rate by the transit time of the extracellular reference, respectively.

Determination of the metabolic clearance of statins using liver microsomes

Rat liver microsomes were prepared from four rats using standard procedures and stored at -80°C until use, and human liver microsomes were purchased from Xenotech LLC (Lenexa, KS). Each statin was incubated with a reaction mixture consisting of liver microsomes (final

concentration, 1 mg/ml) and an NADPH-generating system (0.8 mM NADP⁺, 8 mM glucose-6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, and 3 mM MgCl₂) in the presence of 100 mM phosphate buffer (pH 7.4). After preincubation at 37 °C for 5 min, each statin (final concentration, 0.1 μM) was added to initiate the enzyme reaction. The reaction was terminated at following time points by mixing the reaction mixture with a 4-fold volume of methanol, followed by centrifugation at 15,000g for 10 min at 4 °C. The time points when the reaction was terminated were 0, 5, 15, 30 and 60 min for the metabolic reaction of pitavastatin in rat microsomes, 0, 5, 15, 30, 60, 90 and 120 min for that in human microsomes, and 0, 5, 15 and 30 min for the metabolic reaction of atorvastatin and fluvastatin in rat and human microsomes. The metabolic reaction was continued until the fraction metabolized was greater than 15% so that we could obtain reliable parameters. The actual fractions metabolized at the end of experiment were 30, 25 and 34% (rat microsomes), and 23, 52 and 65% (human microsomes) for pitavastatin, atorvastatin and fluvastatin, respectively.

The supernatant was subjected to LC–MS/MS analysis. The metabolic velocity was calculated as the slope of the natural log (concentration)–time plot. The *in vitro* intrinsic metabolic clearance (CL_{met,int,vitro}) was calculated by dividing initial metabolic velocity by the statin concentration in the incubation buffer corrected by the fraction unbound to liver microsomes. A physiological scaling factor of 44.8 mg protein/g liver (rats) or 48.8 mg protein/g liver (humans) was used for scaling up to the organ level (Naritomi et al., 2001).

Determination of protein binding

Binding of statins to plasma proteins, liver microsomes, or perfusion buffer containing BSA used in the MID study was determined by an ultrafiltration method. Rat plasma was obtained by the centrifugation of blood from male SD rats, and human serum was purchased from Cosmo Bio Co. (Tokyo, Japan). Each statin (final concentration; 5 μM, 0.1 μM, and 50 μM for plasma, microsome, and perfusate, respectively) was added to the protein solution and

incubated at 37 °C for 5 min. The specimen was applied to YM-30 Centrifree devices (Millipore Corporation, Bedford, MA), and the devices were centrifuged at 2000g for 5 min at 37 °C. The fraction unbound was calculated as concentration found in filtrate/total concentration. The concentrations of the drugs in the filtrate and the protein solution before filtration were determined by LC–MS/MS. The adsorption of pravastatin, pitavastatin, and atorvastatin to the filter was negligible, and that of fluvastatin was 19%. The binding of fluvastatin was normalized with respect to the filter blank.

Determination of the blood-to-plasma concentration ratio (R_B)

To determine the R_B values, blood was obtained from male SD rats. Statins (final concentration: 1 μ M) were individually added to the blood samples, and they were incubated together at 37 °C for 5 min. Plasma was prepared by centrifugation of the blood samples (1500g, 5 min). The concentrations of the statins in the plasma samples were determined by LC–MS/MS. R_B values in humans were cited from the previous studies (Tse et al., 1993; Lennernas and Fager, 1997; Regulatory documentation). The unbound fraction in the blood (f_B) was calculated by dividing the unbound fraction in plasma by R_B .

LC–MS/MS analysis

The appropriate standard curves were prepared in the equivalent blank matrix and used for each analysis. High-concentration samples were diluted appropriately with blank matrix. R-122798 (for pravastatin and pitavastatin) and cerivastatin (for atorvastatin and fluvastatin) were used as analytical internal standards.

The LC–MS/MS system consisted of an Alliance 2795 separations module with an autosampler (Waters, Milford, MA) and a Micromass Quattro Ultima tandem quadrupole mass spectrometer with an electron ion spray interface (Waters). The desolvation gas (nitrogen) flow rate was 650 l/h, the cone gas (nitrogen) flow rate was 30 l/h, the source temperature was 150°C and the desolvation temperature was 450°C.

It was operated in a multiple reaction-monitoring mode using negative ion mode. Deprotonated molecular ions were formed using a capillary energy of 3.2 kV and cone energies of 50 V (pravastatin), 45 V (pitavastatin and cerivastatin), 40 V (atorvastatin and fluvastatin). Product ions formed at collision energies of 12 eV (pravastatin, m/z 423.5 \rightarrow 321.2; cerivastatin, m/z 458.5 \rightarrow 396.1), 12 eV (pitavastatin, m/z 420.5 \rightarrow 358.1; R-122798, m/z 409.5 \rightarrow 321.2), 28 eV (atorvastatin, m/z 557.6 \rightarrow 397.2), and 15 eV (fluvastatin, 410.3 \rightarrow 348.2) were monitored. The mobile phase used for high-performance liquid chromatography was 0.1% formic acid/acetonitrile = 73/27 (for pravastatin and pitavastatin) or 55/45 (for atorvastatin and fluvastatin), and the flow rate was 0.4 ml/min. Chromatographic separation was achieved on a C18 column (Capcell Pak C18 MG-II column, 50 \times 2 mm; particle size, 3 μ m; Shiseido, Tokyo, Japan).

Eight points calibration curves were generated by plotting the peak area ratios of analyte/internal standard against the nominal analyte concentrations using linear regression with $1/(\text{area ratio})^2$ weighting. The typical R-squared value of the calibration curves was 0.997 to 0.999. The concentration range was 1 to 1,000 nM for atorvastatin and 3 to 3,000 nM for the other statins. The back-calculated concentrations of all the calibration standards were to be within 15% of their individual nominal concentrations [\pm 20% at the lower limit of quantitation]. Intraday and interday variability for the quantification of statins was less than 15%.

Pharmacokinetic analysis in rats

Pharmacokinetic parameters were calculated using noncompartmental analysis. Area under the plasma concentration–time curve (AUC_p) was calculated using the trapezoidal rule with extrapolation to infinity, and total blood clearance ($CL_{tot,B}$) was estimated as $\text{dose}/(AUC_p \times R_B)$. $CL_{tot,B}$ was regarded as the hepatic clearance (CL_H) because the urinary excretion of all statins in male SD rats was negligible. Hepatic availability (F_H) of pitavastatin, atorvastatin, and fluvastatin was calculated from the following equation:

$$CL_H = Q_H \cdot (1 - F_H) \quad (3)$$

where Q_H represents the hepatic blood flow. F_H of pravastatin could not be estimated accurately from Eq. 3, because CL_H of pravastatin was hepatic blood flow limited, in other words, F_H was extremely small. Therefore, F_H of pravastatin was obtained by dividing its bioavailability (Watanabe et al., 2009) by the fraction absorbed (Komai et al., 1992), assuming negligible metabolism in the small intestine. $CL_{int,all,vivo}$ was calculated from the following equations using a dispersion model (Roberts and Rowland, 1986).

$$F_H = \frac{4a}{(1+a)^2 \cdot \exp\{(a-1)/2/D_N\} - (1-a)^2 \cdot \exp\{-(a+1)/2/D_N\}} \quad (4)$$

$$a = (1 + 4R_N \cdot D_N)^{1/2} \quad (5)$$

$$R_N = f_B \cdot \frac{CL_{int,all,vivo}}{Q_H} \quad (6)$$

The hepatic blood flow rate was set at 50–80 ml/min/kg for rats and at 17–25.5 ml/min/kg for humans, and D_N was set at 0.17. A physiological scaling factor of 41.2 g liver/kg body weight (rats) or 24.1 g liver/kg body weight (humans) was used for scaling down to the organ level.

Pharmacokinetic analysis in humans

The availability in the liver (F_H) of pravastatin and atorvastatin were calculated using Eq. 3 using the plasma concentration and urinary excretion data after intravenous administration in the clinical studies and f_B values (Singhvi et al., 1990; Atorvastatin regulatory documentation). In the case of fluvastatin, F_H was calculated by dividing its bioavailability (0.33) by the fraction absorbed in humans (0.9), because its hepatic clearance (16 ml/min/kg) was close to the hepatic blood-flow rate (Tse et al., 1992; Lindahl et al., 1996). F_H of pitavastatin was obtained from the

following equation (Eq. 7) using the plasma concentration data after oral administration in humans (Ando et al., 2005; Package Insert) and fraction absorbed (F_a) in rats (0.83, (Kimata et al., 1998)), assuming no interspecies differences in F_a and negligible metabolism in the small intestine:

$$F_H = \frac{Q_H}{F_a \cdot CL_{oral} + Q_H} \quad (7)$$

where CL_{oral} is blood clearance after oral administration. Subsequently, $CL_{int,all,vivo}$ of each statin was calculated from Eqs. (4)–(6).

Results

Uptake clearances of statins determined using freshly isolated rat hepatocytes and cryopreserved human hepatocytes

The uptake clearances ($PS_{\text{inf,vitro}}$) of the statins were determined using rat and human hepatocytes (Fig. 1). The uptake clearance was markedly lower using the addition of excess amounts of the statins in both rat and human hepatocytes. $PS_{\text{inf,vitro}}$ determined at 0.1 μM are scaled up to the in vivo value per unit liver weight using the following physiological scaling factors, 41.2 g liver/kg, 1.2×10^8 cells/g liver for comparison with the corresponding $PS_{\text{inf,MID}}$. $PS_{\text{inf,vitro}}$ of statins in rats were almost similar or somewhat lower than $PS_{\text{inf,MID}}$ (Table 3).

Determination of the in vivo intrinsic hepatic clearance of statins in rats

Figure 2 shows time profiles of the plasma concentrations and the cumulative amount of statins excreted into the bile following intravenous administration. Approximately 50% of the dose was recovered in the bile after the administration of pitavastatin and atorvastatin, while fluvastatin was slightly excreted into the bile as unchanged form. Urinary excretion of all the statins was negligible. The pharmacokinetic parameters of the statins were determined by noncompartmental analysis (Table 1). The plasma/serum unbound fraction and blood-to-plasma concentration ratio (R_B) of each statin were also measured, and the findings are summarized in Table 2.

Determination of the *in situ* intrinsic hepatic uptake clearance of statins

The intrinsic hepatic uptake clearance ($PS_{\text{inf,MID}}$) was determined by the multiple indicator dilution method using FD-4 as an extracellular space marker. The natural logarithm of the ratio of the concentration of FD-4 to that of each statin in the outflow (ratio plot) is given as a function of time in Fig. 3. The intrinsic hepatic uptake clearance ($PS_{\text{inf,MID}}$) of statins was determined from the slope of the plot and unbound fraction in the perfusate (Tables 2 and 3).

For pitavastatin, in order to validate the unbound uptake clearance, $PS_{inf,MID}$ was determined using the perfusion buffers containing 1.5% or 3% BSA. The unbound fractions of pitavastatin in the presence of 1.5% and 3% BSA were 0.0859 and 0.0489, respectively, and $PS_{inf,MID}$ was similar (74.1 ± 25.1 and 91.5 ± 8.5 ml/min per g liver (mean \pm S.E.), respectively).

Comparison of intrinsic clearances in the hepatic elimination of statins by rats

Intrinsic clearances related to the hepatic clearance of statins, such as $PS_{inf,MID}$, $PS_{inf,vitro}$, $CL_{met,int,vitro}$, and $CL_{int,all,vivo}$, by rats are summarized in Table 3. All parameters were expressed as the value per unit weight of the liver. $CL_{int,all,vivo}$ was determined from Eqs. (4)–(6) using F_H and f_B of each statin (Tables 1 and 2) and Q_H (1.21–1.94 ml/min/g liver). $PS_{inf,MID}$ and $PS_{inf,vitro}$ of statins were similar to $CL_{int,all,vivo}$, while $CL_{met,int,vitro}$ was much lower than $CL_{int,all,vivo}$ (Fig. 4).

Comparison of intrinsic clearances in the hepatic clearance of statins by humans

Intrinsic clearances of statins by humans are summarized in Table 4. All parameters are expressed as the value per the unit weight of the liver except for the scaling factor. The uptake clearances determined by the in vitro model were extrapolated to in vivo clearances using physiological- and drug-related scaling factors, and the latter scaling factor was defined as the ratio of the in situ to in vitro uptake clearances for each statin in rats. As observed in rats, the predicted uptake clearances ($PS_{inf,vivo,predicted}$) were similar to $CL_{int,all,vivo}$ obtained from the clinical studies. In contrast, the $CL_{met,int,vitro}$ of atorvastatin and fluvastatin were markedly low to account for $CL_{int,all,vivo}$ (Fig. 4), although these statins are mainly eliminated from the liver through metabolism by CYP.

Discussion

Previously, based on pharmacokinetic analyses, we proposed that the uptake is the rate-determining process in the overall hepatic elimination of pravastatin (Watanabe et al., 2009). The present study examined the rate-determining step in the overall hepatic elimination of other statins with different elimination mechanisms: biliary excretion for pitavastatin, and CYP-mediated metabolism for atorvastatin and fluvastatin.

Consistent with previous studies (Hirano et al., 2004), the uptake of pitavastatin by human hepatocytes was saturable (Fig.1). In addition, uptake of atorvastatin and fluvastatin was also saturable in human hepatocytes (Fig.1). Saturable uptake of atorvastatin is in a good agreement with the clinical report in which co-administration of rifampicin, an inhibitor of OATP1B1, greatly enhanced the systemic exposure of atorvastatin, and the systemic exposure of atorvastatin is affected by the genotypes of OATP1B1 (Lau et al., 2007; He et al., 2009). Although an in vitro study using cDNA transfectants showed that fluvastatin is a substrate of OATP1B1 (Kopplow et al., 2005; Noe et al., 2007), the systemic exposure of fluvastatin was independent of the OATP1B1 genotypes (Niemi et al., 2006), suggesting that a transporter distinct from OATP1B1 may play a major role in the hepatic uptake of fluvastatin. An in vitro study using cDNA transfectants showed that fluvastatin is also a substrate of other hepatic uptake transporters, such as OATP1B3 and OATP2B1 (Kopplow et al., 2005; Noe et al., 2007). As observed for other statins, variation of the uptake transport activities has a reciprocal relationship to the blood concentration of fluvastatin, and particularly, reduction of the uptake activity will increase the risk of an adverse reaction. Because of its negligible urinary excretion of fluvastatin, the variation will have only a minimal impact on the liver concentration (Watanabe et al., 2009), and pharmacological response.

The rate-determining process was identified in rats by comparing the in vivo intrinsic hepatic clearance and uptake clearance. To obtain intrinsic hepatic clearance, the

pharmacokinetics of pitavastatin, atorvastatin, and fluvastatin were examined in rats. Considering the recovery of the unchanged forms in the bile, biliary excretion and metabolism make a similar contribution to the intrinsic hepatic clearance of pitavastatin and atorvastatin, while hepatic metabolism is the predominant pathway for elimination of fluvastatin in rats. The uptake clearance ($PS_{\text{inf,MID}}$) of the statins was found to be similar to their corresponding intrinsic hepatic clearances ($CL_{\text{int,all,vivo}}$) (Table 3 and Fig. 4). Namely, uptake is the rate-determining process in the hepatic elimination of the statins. In contrast, intrinsic sequestration clearances (metabolism for fluvastatin, biliary excretion and metabolism for atorvastatin and pitavastatin) were negligibly low accounting for the $CL_{\text{int,all,vivo}}$ (Table 3 and Fig. 4). This poor predictability of the in vivo intrinsic hepatic clearance of pitavastatin, atorvastatin, and fluvastatin from a study of in vitro metabolism is likely the result of active uptake from the blood in the sinusoidal membrane.

The rate-determining process in the hepatic elimination of the statins was also examined in humans. Previously, we introduced the rat scaling factor to extrapolate in vitro uptake clearance of pravastatin determined using human hepatocytes to the in vivo clearance, which provided reasonable parameters to reproduce the plasma concentration–time profiles of pravastatin following intravenous and oral administration (Singhvi et al., 1990; Watanabe et al., 2009). In this study, we determined the scaling factor for each statin in rats by comparing their in situ and in vitro uptake clearances. The scaling factors of statins appear compound dependent, ranging from 1.7 to 4.5 (Table 4). The scaling factor of the statins (pravastatin, atorvastatin and pitavastatin) the hepatic uptake of which is mediated mainly by OATP1B1 is roughly 2, while that of fluvastatin the hepatic uptake of which is mediated by the transporter distinct from OATP1B1 is 2-fold greater. Therefore, it can be speculated that the scaling factor is transporter-dependent. To support this speculation, accumulation of IVIVE data for the transporters is absolutely essential. Because 1) the in vivo uptake clearance predicted for humans was in the range of $CL_{\text{int,all,vivo}}$ (Table 4), and 2) $CL_{\text{met,int,vitro}}$ of atorvastatin and fluvastatin determined using

human liver microsomes was below $CL_{int,all,vivo}$, uptake is the most likely rate-determining process in the hepatic elimination of the statins in humans. Lau, et al. (2007) also suggested that hepatic uptake was important for systemic exposure of atorvastatin based on the clinical drug-drug interaction study between atorvastatin and a potent inhibitor of OATPs, rifampicin. Thus, impact of the variation of the sequestration clearance (metabolism or biliary excretion) caused by drug–drug interactions or genetic polymorphisms depends on the rate-determining process and will be smaller for these statins compared with drugs that achieve a rapid equilibrium. Indeed, the increase (2.5- to 3-fold) in the AUC of atorvastatin caused by concomitant use of itraconazole, a potent CYP3A4 inhibitor, was less remarkable than for other CYP3A4 substrates such as midazolam and triazolam (5 to 10-fold increase) (Venkatakrishnan et al., 2000; Shitara and Sugiyama, 2006). BCRP genotypes produce no significant interindividual variation of the systemic exposure of pitavastatin (Ieiri et al., 2007), although they play a predominant role in mice (Hirano et al., 2005). It should be noted that, irrespective of the rate-determining process, the sequestration clearance is the predominant factor determining the liver concentration of the statins (Watanabe et al., 2009). Thus, inhibition of CYP3A4 or BCRP polymorphisms results in a significant increase in the liver concentration of atorvastatin and pitavastatin, respectively, leading to the enhancement of their pharmacological action. To validate the prediction of the rate-determining process of these statins, information regarding the tissue concentration–time profile is necessary. Clinical studies using positron emission tomography/single photon emission computed tomography, will allow an advancement to improve the predictability of pharmacokinetic parameters.

To determine the intrinsic hepatic clearance that comprises uptake, sinusoidal efflux, and metabolism, Soars et al. (2007) proposed a “media loss” assay using isolated hepatocytes. Using this method, an intrinsic clearance can be determined from the concentration–time profile of drugs in incubation media and the initial amount of drugs applied. Theoretically, the method must be able to provide a reliable overall hepatic intrinsic clearance. However, as described in

the report by Soars et al. (2007), this method considerably underestimates the in vivo overall intrinsic clearance (with an average 16-fold error), possibly because of reduced activities of transporters and/or enzymes during the relatively long-term incubation. Therefore, at present, separate determination of the uptake and metabolic clearances will provide more reliable parameters to predict the intrinsic hepatic clearance using rat scaling factors.

The present study found that the underestimation of in vivo intrinsic hepatic clearance of the statins in the in vitro–in vivo extrapolation of metabolic clearance is because of active transport in the uptake process. Kinetic analyses demonstrated that uptake is the rate-determining process in the hepatic elimination of the statins in rats, which likely holds true in humans. In vitro–in vivo extrapolation of the uptake clearance using a human hepatocytes model and scaling factors determined in rats should be effective for predicting in vivo intrinsic hepatic clearance of drugs when transporter(s) are involved in the hepatic uptake.

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References

- Akita H, Suzuki H and Sugiyama Y (2002) Sinusoidal efflux of taurocholate correlates with the hepatic expression level of Mrp3. *Biochem Biophys Res Commun.* **299**:681-687.
- Ando H, Tsuruoka S, Yanagihara H, Sugimoto K, Miyata M, Yamazoe Y, Takamura T, Kaneko S and Fujimura A (2005) Effects of grapefruit juice on the pharmacokinetics of pitavastatin and atorvastatin. *Br J Clin Pharmacol.* **60**:494-497.
- Bidstrup TB, Bjornsdottir I, Sidelmann UG, Thomsen MS and Hansen KT (2003) CYP2C8 and CYP3A4 are the principal enzymes involved in the human in vitro biotransformation of the insulin secretagogue repaglinide. *Br J Clin Pharmacol.* **56**:305-314.
- Fischer V, Johanson L, Heitz F, Tullman R, Graham E, Baldeck JP and Robinson WT (1999) The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor fluvastatin: effect on human cytochrome P-450 and implications for metabolic drug interactions. *Drug Metab Dispos.* **27**:410-416.
- He YJ, Zhang W, Chen Y, Guo D, Tu JH, Xu LY, Tan ZR, Chen BL, Li Z, Zhou G, Yu BN, Kirchheiner J and Zhou HH (2009) Rifampicin alters atorvastatin plasma concentration on the basis of SLCO1B1 521T>C polymorphism. *Clin Chim Acta* **14**:14.
- Hirano M, Maeda K, Matsushima S, Nozaki Y, Kusuha H and Sugiyama Y (2005) Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin. *Mol Pharmacol.* **68**:800-807.
- Hirano M, Maeda K, Shitara Y and Sugiyama Y (2004) Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in humans. *J Pharmacol Exp Ther.* **311**:139-146.
- Ieiri I, Suwannakul S, Maeda K, Uchimaru H, Hashimoto K, Kimura M, Fujino H, Hirano M, Kusuha H, Irie S, Higuchi S and Sugiyama Y (2007) SLCO1B1 (OATP1B1, an uptake transporter) and ABCG2 (BCRP, an efflux transporter) variant alleles and pharmacokinetics of pitavastatin in healthy volunteers. *Clin Pharmacol Ther.* **82**:541-547.
- Iwai M, Suzuki H, Ieiri I, Otsubo K and Sugiyama Y (2004) Functional analysis of single nucleotide polymorphisms of hepatic organic anion transporter OATP1B1 (OATP-C). *Pharmacogenetics* **14**:749-757.
- Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, Ishizaki T, Green CE, Tyson CA and Sugiyama Y (1997) Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. *Pharmacol Ther* **73**:147-171.
- Jacobsen W, Kuhn B, Soldner A, Kirchner G, Sewing KF, Kollman PA, Benet LZ and Christians U (2000) Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin. *Drug Metab Dispos.* **28**:1369-1378.
- Kilford PJ, Stringer R, Sohal B, Houston JB and Galetin A (2008) Prediction of drug clearance by glucuronidation from in vitro data: Use of combined P450 and UGT cofactors in alamethicin activated human liver microsomes. *Drug Metab Dispos* **2**:2.

- Kimata H, Fujino H, Koide T, Yamada Y, Tsunenari Y and Yanagawa Y (1998) Studies on the Metabolic Fate of NK-104, a New Inhibitor of HMG-CoA Reductase (1): Absorption, Distribution, Metabolism and Excretion in Rats. *Xenobiotic Metab. Dispos.* **13**:484-498.
- Kirchheiner J, Kudlicz D, Meisel C, Bauer S, Meineke I, Roots I and Brockmoller J (2003) Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. *Clin Pharmacol Ther.* **74**:186-194.
- Komai T, Kawai K, Tokui T, Tokui Y, Kuroiwa C, Shigehara E and Tanaka M (1992) Disposition and metabolism of pravastatin sodium in rats, dogs and monkeys. *Eur J Drug Metab Pharmacokinet* **17**:103-113.
- Kopplow K, Letschert K, Konig J, Walter B and Keppler D (2005) Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. *Mol Pharmacol.* **68**:1031-1038.
- Kusuhara H and Sugiyama Y (2009) In vitro-in vivo extrapolation of transporter-mediated clearance in the liver and kidney. *Drug Metab Pharmacokinet* **24**:37-52.
- Lau YY, Huang Y, Frassetto L and Benet LZ (2007) effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. *Clin Pharmacol Ther.* **81**:194-204.
- Lennernas H and Fager G (1997) Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. *Clin Pharmacokinet.* **32**:403-425.
- Lindahl A, Sandstrom R, Ungell AL, Abrahamsson B, Knutson TW, Knutson L and Lennernas H (1996) Jejunal permeability and hepatic extraction of fluvastatin in humans. *Clin Pharmacol Ther.* **60**:493-503.
- Maeda K, Ieiri I, Yasuda K, Fujino A, Fujiwara H, Otsubo K, Hirano M, Watanabe T, Kitamura Y, Kusuhara H and Sugiyama Y (2006) Effects of organic anion transporting polypeptide 1B1 haplotype on pharmacokinetics of pravastatin, valsartan, and temocapril. *Clin Pharmacol Ther* **79**:427-439.
- Miyauchi S, Sawada Y, Iga T, Hanano M and Sugiyama Y (1993) Comparison of the hepatic uptake clearances of fifteen drugs with a wide range of membrane permeabilities in isolated rat hepatocytes and perfused rat livers. *Pharm Res.* **10**:434-440.
- Naritomi Y, Terashita S, Kimura S, Suzuki A, Kagayama A and Sugiyama Y (2001) Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans. *Drug Metab Dispos* **29**:1316-1324.
- Niemi M, Pasanen MK and Neuvonen PJ (2006) SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. *Clin Pharmacol Ther* **80**:356-366.
- Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, Urae A, Higuchi S, Otsubo K and Sugiyama Y (2003) Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* **73**:554-565.

- Noe J, Portmann R, Brun ME and Funk C (2007) Substrate-dependent drug-drug interactions between gemfibrozil, fluvastatin and other organic anion-transporting peptide (OATP) substrates on OATP1B1, OATP2B1, and OATP1B3. *Drug Metab Dispos.* **35**:1308-1314.
- Nozawa T, Minami H, Sugiura S, Tsuji A and Tamai I (2005) Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. *Drug Metab Dispos* **33**:434-439.
- Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos.* **27**:1350-1359.
- Roberts MS and Rowland M (1986) A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations. *J Pharmacokinet Biopharm.* **14**:227-260.
- Shitara Y and Sugiyama Y (2006) Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. *Pharmacol Ther.* **112**:71-105.
- Singhvi SM, Pan HY, Morrison RA and Willard DA (1990) Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. *Br J Clin Pharmacol* **29**:239-243.
- Soars MG, Grime K, Sproston JL, Webborn PJ and Riley RJ (2007) Use of hepatocytes to assess the contribution of hepatic uptake to clearance in vivo. *Drug Metab Dispos.* **35**:859-865.
- Stringer R, Nicklin PL and Houston JB (2008) Reliability of human cryopreserved hepatocytes and liver microsomes as in vitro systems to predict metabolic clearance. *Xenobiotica.* **38**:1313-1329.
- Tirona RG, Leake BF, Merino G and Kim RB (2001) Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* **276**:35669-35675.
- Tse FL, Jaffe JM and Troendle A (1992) Pharmacokinetics of fluvastatin after single and multiple doses in normal volunteers. *J Clin Pharmacol.* **32**:630-638.
- Tse FL, Nickerson DF and Yardley WS (1993) Binding of fluvastatin to blood cells and plasma proteins. *J Pharm Sci.* **82**:942-947.
- Ueda K, Kato Y, Komatsu K and Sugiyama Y (2001) Inhibition of biliary excretion of methotrexate by probenecid in rats: quantitative prediction of interaction from in vitro data. *J Pharmacol Exp Ther.* **297**:1036-1043.
- Venkatakrishnan K, von Moltke LL and Greenblatt DJ (2000) Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. *Clin Pharmacokinet* **38**:111-180.
- Watanabe T, Kusuhara H, Maeda K, Shitara Y and Sugiyama Y (2009) Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther.* **328**:652-662.

- Yamazaki M, Akiyama S, Nishigaki R and Sugiyama Y (1996) Uptake is the rate-limiting step in the overall hepatic elimination of pravastatin at steady-state in rats. *Pharm Res* **13**:1559-1564.
- Yamazaki M, Suzuki H, Hanano M, Tokui T, Komai T and Sugiyama Y (1993) Na(+)-independent multispecific anion transporter mediates active transport of pravastatin into rat liver. *Am J Physiol* **264**:G36-44.

Footnotes

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Legends for figures

Fig. 1. Uptake clearance of statins in isolated human and rat hepatocytes.

Uptake clearance of four statins by hepatocytes determined at 37 °C at two concentrations (closed bar, 0.1 μ M; open bar, 100 μ M) by the oil filtration method. Cryopreserved human hepatocytes (three independent batches depicted as Lot A, -B and -C) and freshly isolated rat hepatocytes were used in the determinations. Cells were incubated with statins for 0.5, 1.5, and 2.5 min; subsequently, reactions were terminated by rapid separation of the cells from the uptake buffer using centrifugation. The uptake is represented by the amount associated with the cell specimens divided by the statin concentrations in the uptake buffer. Data represent the mean \pm S.E. ($n = 3$).

Fig. 2. Plasma concentration–time profiles (upper panels) and cumulative biliary excretion (lower panels) of statins after intravenous administration to male SD rats.

Male SD rats were given statins, pitavastatin (1 μ mol/kg, left), atorvastatin (1 μ mol/kg, middle), and fluvastatin (0.5 μ mol/kg, right), intravenously. The plasma concentrations were determined over 240 min following administration for pitavastatin, 180 min for atorvastatin, and 240 min for fluvastatin. Bile was collected from the common bile duct via an indwelling cannula, and the cumulative amount of biliary excretion was determined. Data represent the mean \pm S.E. ($n = 3$).

Fig. 3. Time profiles of the natural logarithm of the concentration ratio of FD-4 to statins in the outflow.

After 10 min preperfusion, Each statin (50 μ M) and FD-4 (100 μ M), an extracellular reference, were injected into the portal vein. After injection, the total effluent from the hepatic vein was collected at 1 s intervals for 10 s. For pitavastatin, the injected solution contained 3% (●) or 1.5% (○) BSA. Data represent the mean \pm S.E. ($n = 3$).

Fig. 4. Comparison of the hepatic overall intrinsic clearance of statins with the hepatic uptake clearance or metabolic clearance.

In vivo hepatic overall intrinsic clearances of statins in rats and humans are plotted against the uptake clearance or metabolic clearance. ●, predicted uptake clearance in humans; ○, metabolic clearance determined using human liver microsomes; ■, uptake clearance in rats determined using a MID method; □, metabolic clearance determined using rat liver microsomes. The straight line indicates a 1:1 correlation. Each point represents the mean \pm S.E. ($n = 3$).

Table 1. Pharmacokinetic parameters of statins in rats

	Dose	CL_{tot,B}	Biliary Excretion	Urinary Excretion	F_H
	<i>μmol/kg</i>	<i>ml/min/kg</i>	<i>% of dose</i>	<i>% of dose</i>	
Pravastatin ^a	0.5	62	43	< 4	0.014
Pitavastatin	1	28	57	< 0.1	0.44–0.65 ^b
Atorvastatin	1	35	50	< 0.1	0.29–0.56 ^b
Fluvastatin	0.5	42	9	< 0.1	0.17–0.48 ^b

^a Watanabe et al., 2009.

^b Estimated from Eq. (3) using Q_H (50 to 80 ml/min/kg).

Table 2. Blood-to-plasma concentration ratio (R_B) and unbound fraction in the perfusion buffer, rat plasma and human serum, and blood unbound fraction (f_B) for each statin

	Unbound fraction					R _B		f _B	
	Perfusion Buffer	Rat microsome	Human microsome	Rat Plasma	Human Serum	Rat	Human ^b	Rat	Human
Pravastatin	0.683	N.D.	N.D.	0.676	0.554	0.59^a	0.56	1.2	0.99
Pitavastatin	0.0489	0.418	0.432	0.0134	0.00523	0.65	0.58	0.021	0.0090
Atorvastatin	0.0817	0.557	0.405	0.0567	0.0511	1.2	0.61	0.047	0.084
Fluvastatin	0.0311	0.234	0.308	0.00986	0.00368	0.53	0.52	0.019	0.0071

^a Yamazaki et al., 1996b.

^b Tse et al. (1993); Lennernas and Fager (1997); Regulatory documentation.

N.D., not determined

Table 3. Intrinsic clearances of statins related to their hepatic clearance in rats

All intrinsic clearances are scaled up to the *in vivo* clearance values per unit liver weight using the following physiological scaling factors: 41.2 g liver/kg, 1.2×10^8 cells/g liver, and 44.8 mg microsomal protein/g liver. Data represent the mean \pm S.E. ($n = 3$).

	Uptake Clearance			Metabolic Clearance			Overall Intrinsic Clearance		
	PS _{inf,MID} ^a		PS _{inf,vitro} ^b	CL _{met,int,vitro} ^c		CL _{int,all,vivo} ^d			
				ml/min/g liver					
Pravastatin	6.48	\pm	0.06	2.59	\pm	0.14	0.793^e	\pm	0.020
Pitavastatin	91.5	\pm	8.5	53.3	\pm	8.3	0.619	\pm	0.434
Atorvastatin	42.1	\pm	5.3	23.1	\pm	2.5	0.910	\pm	0.056
Fluvastatin	126	\pm	12	29.2	\pm	2.0	2.71	\pm	0.24

^a Determined by the MID analysis.

^b Determined using rat hepatocytes at 0.1 μ M.

^c Determined using rat liver microsomes.

^d Determined using the dispersion model with F_H, f_B, and Q_H (1.21–1.94 mL/min/g liver).

^e Determined using rat liver S9 (Watanabe et al., 2009).

Fig. 1

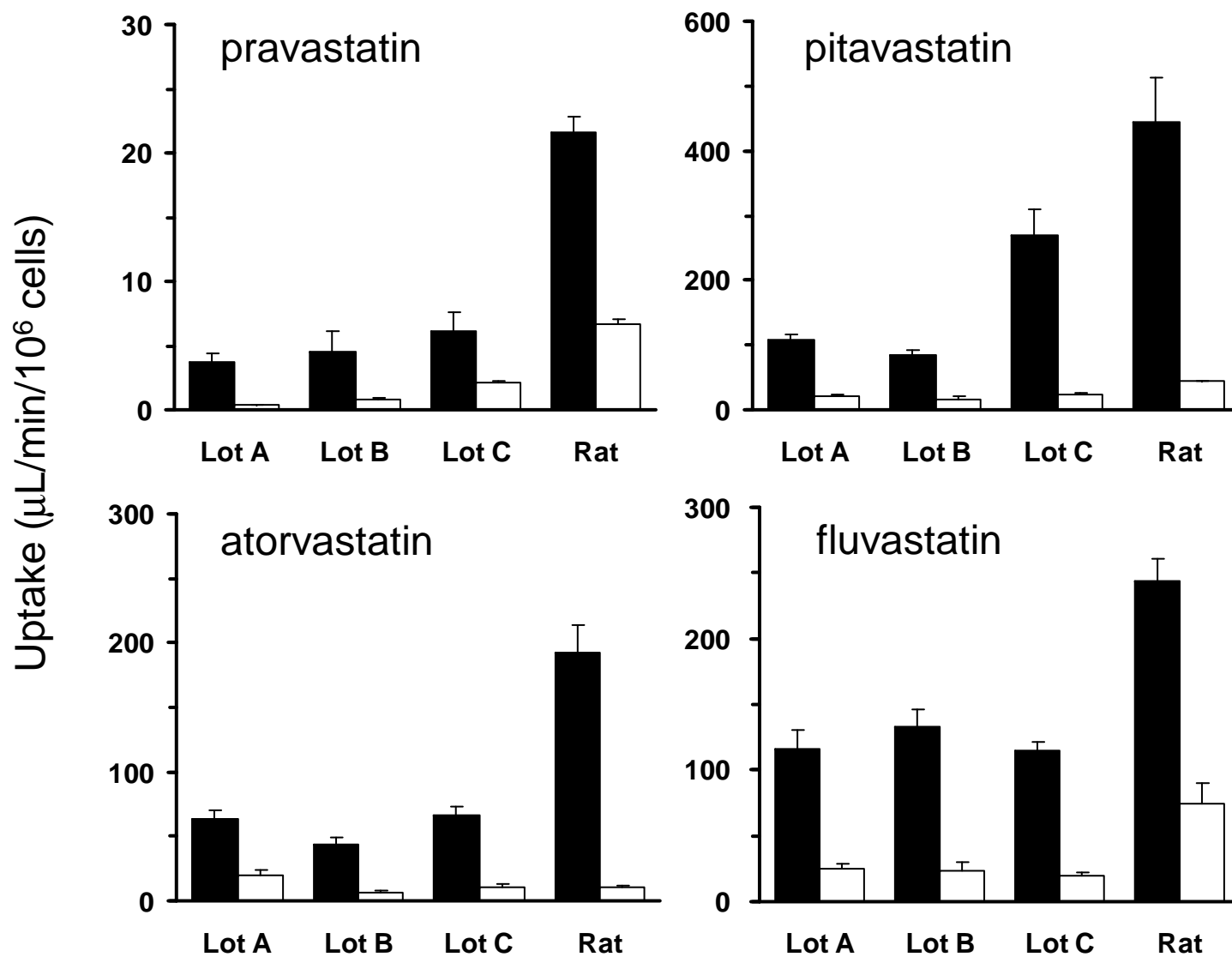


Fig. 2

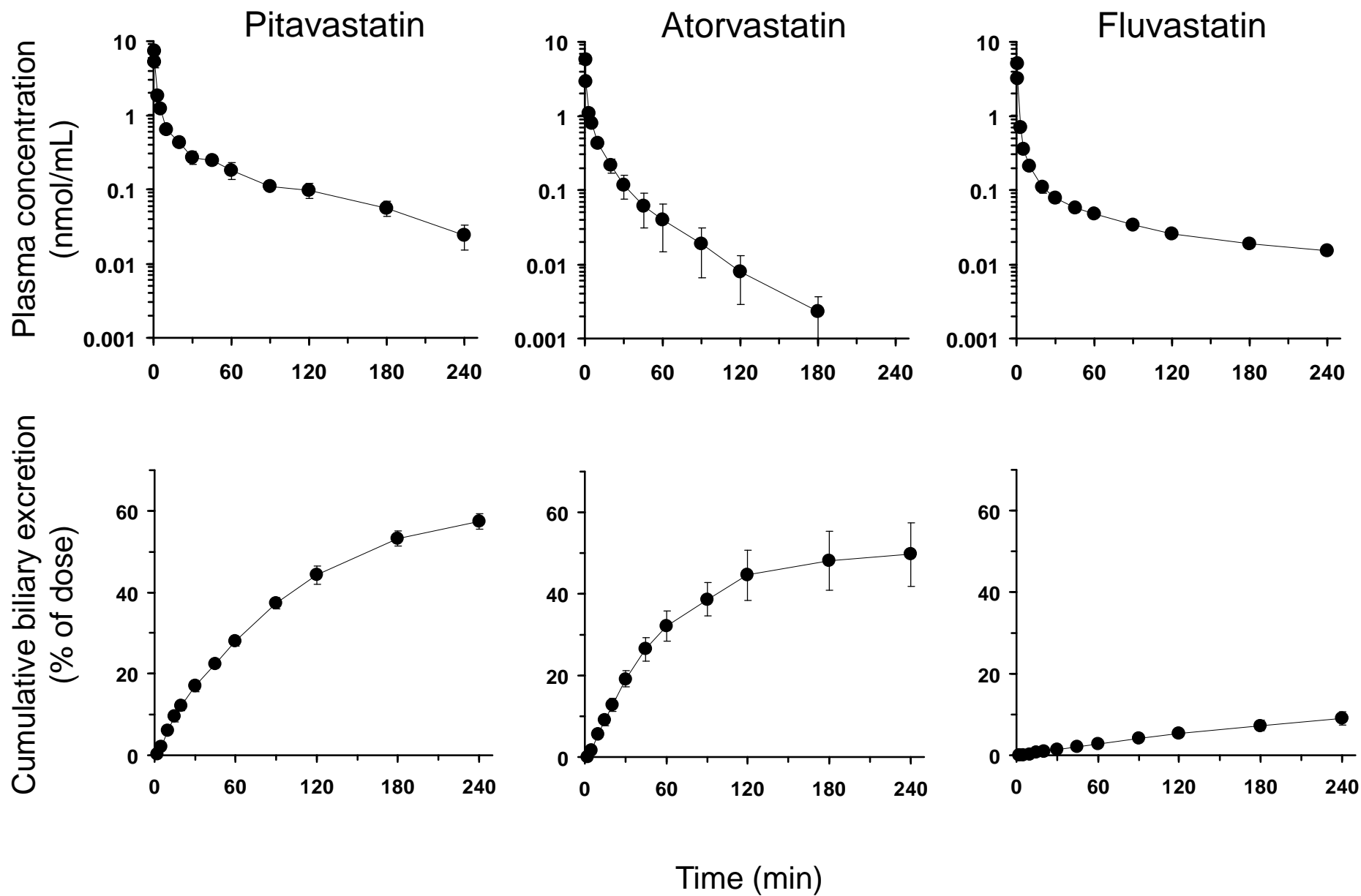


Fig. 3

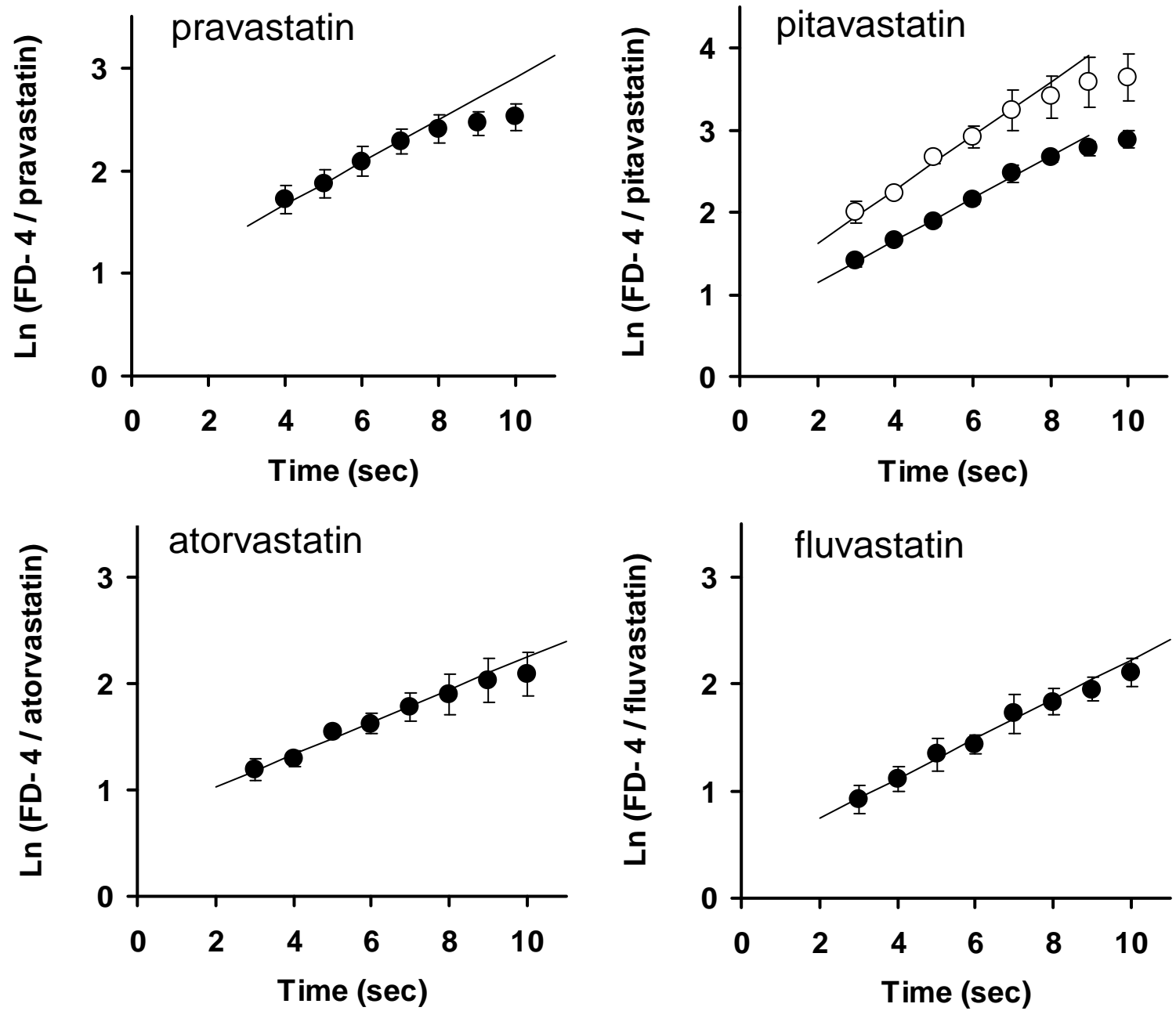


Fig. 4

