Physiologically Based Pharmacokinetic Modeling to Predict Transporter-Mediated Clearance and Distribution of Pravastatin in Humans

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ABSTRACT

Hepatobiliary excretion mediated by transporters, organic anion-transporting polypeptide (OATP) 1B1 and multidrug resistance-associated protein (MRP) 2, is the major elimination pathway of an HMG-CoA reductase inhibitor, pravastatin. The present study examined the effects of changes in the transporter activities on the systemic and liver exposure of pravastatin using a physiologically based pharmacokinetic model. Scaling factors, determined by comparing in vivo and in vitro parameters of pravastatin in rats for the hepatic uptake and canalicular efflux, were obtained. The simulated plasma and liver concentrations and biliary excretion profiles were very close to the observed data in rats under linear and nonlinear conditions. In vitro parameters, determined in human cryopreserved hepatocytes and canalicular membrane vesicles, were extrapolated to in vivo parameters using the scaling factors obtained in rats. The simulated plasma concentrations of pravastatin were close to the reported values in humans. Sensitivity analyses showed that changes in the hepatic uptake ability altered the plasma concentration of pravastatin markedly but had a minimal effect on the liver concentration, whereas changes in the ability of canalicular efflux altered the liver concentration of pravastatin markedly but had a small effect on the plasma concentration. In conclusion, the model allows the prediction of the disposition of pravastatin in humans. The present study suggests that changes in the OATP1B1 activities may have a small and a large impact on the therapeutic efficacy and side effect (myopathy) of pravastatin, respectively, whereas those in the MRP2 activities may have opposite impacts (i.e., large and small impacts on the therapeutic efficacy and side effect).

Predicting the disposition of drugs in humans, particularly in the early stages of drug development, has been a critical issue in selecting the proper candidate drugs because the exposure of drugs to target organs is the major factor determining their pharmacological and/or toxicological activity. Human liver microsomes allow the reliable prediction of the metabolic clearance of drugs in humans (Rane et al., 1977; Iwatsubo et al., 1997; Obach, 1999; Naritomi et al., 2001). Biliary excretion, another hepatic elimination pathway, is the major systemic elimination pathway, particularly for amphipathic anionic drugs such as HMG-CoA reductase inhibitors (statins) and angiotensin II receptor antagonists. Because multiple transporters on the sinusoidal and canalicular membranes are involved, it is necessary to separately determine three kinetic parameters: 1) uptake, 2) sinusoidal efflux, and 3) canalicular efflux, to predict biliary clearance with regard to the plasma concentration (Giacomini and Sugiyama, 2005; Shitara et al., 2006a). The uptake clearance determined in freshly isolated rat hepatocytes correlates well with that determined with the multiple indicator dilution method (Miyauchi et al., 1993), and rat hepatocytes are reported to be a useful tool for predicting the hepatic clearance of drugs with significant hepatic uptake (Soars et al., 2007). Although cryopreserved human hepatocytes and canalicular membrane vesicles (CMVs) are commercially available, their usefulness in predicting in vivo hepatic up-
Materials and Methods

Materials

\(^{3}H\)Pravastatin (45.5 Ci/mmol), unlabeled pravastatin, and a pravastatin analog, R-122798, were provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Cryopreserved human hepatocytes and human liver S9 fractions were purchased from In Vitro Technologies (Baltimore, MD). Human liver S9 fractions were also purchased from Xenotech, LLC (Lenexa, KS) and Tissue Transformation Technology (Edison, NJ). All other chemicals and reagents were of analytical grade and were readily available from commercial sources.

Animals

Male Sprague-Dawley rats (6–7 weeks old) were purchased from Nippon SLC (Hamamatsu, 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 carried out in accordance with the guidelines of the Institutional Animal Care Committee, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.

Animal Experiments

Male Sprague-Dawley rats, weighing approximately 250 to 320 g, were used throughout the 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 or the duodenum was cannulated with a polyethylene catheter (SP-31) for the administration of pravastatin. Each rat was placed in a Bollman cage and allowed to recover from the anesthesia before the experiments were continued. The rats were given pravastatin intravenously at 0.2, 1, 10, 50, or 200 mg/kg or intraduodenally at 20 mg/kg. 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. After the last blood sample had been taken, each rat was killed, and the liver, kidney, brain, lungs, and skeletal muscle were excised immediately for the tissue distribution study. The tissues were weighed and flash frozen in liquid nitrogen. All the samples were stored at −20°C until quantification.

Transport Study Using Human Cryopreserved Hepatocytes

This experiment was performed as described previously (Shitara et al., 2003). In brief, immediately before the study, the hepatocytes were thawed at 37°C. After they had been washed twice with ice-cold Krebs-Henseleit buffer, the cells were suspended in Krebs-Henseleit buffer to a cell density of 1.0 × 10^6 viable cells/ml for the uptake study. After preincubation of the cells (1.2 × 10^6 cells/reaction) at 37°C for 3 min, drug uptake was initiated by the addition of labeled and unlabeled substrates to the cell suspension. The reaction was terminated after 0.5 or 2 min by separating the cells from the substrate solution. For this purpose, an aliquot of 100 μl of incubation mixture was placed in a centrifuge tube (450 μl) containing 50 μl of 2 N NaOH under a layer of 100 μl of oil (density = 1.015, a mixture of silicone oil and mineral oil; Sigma-Aldrich, St. Louis, MO). The sample tube was centrifuged for 10 s in a tabletop centrifuge (10,000g; Beckman Microfuge E; Beckman Coulter, Fullerton, CA). After overnight incubation in alkali to dissolve the hepatocytes, the centrifuge tube was cut, and each phase was transferred to a scintillation vial. The phase containing the dissolved cells was neutralized with 50 μl of 2 N HCl, mixed with scintillation cocktail, and its radioactivity was measured in a liquid scintillation counter (LS6000SE; Beckman Coulter). The time course for the uptake of \(^{3}H\)pravastatin into hepatocytes was expressed as the uptake volume (microliters per 10^6 viable cells) of the radioactivity taken up into the cells (disintegrations per minute per 10^6 cells) divided by the concentration of radioactivity in the incubation buffer (disintegrations per minute per microliter). The initial uptake velocity of \(^{3}H\)pravastatin was calculated from the slopes of the uptake volume versus time plots.
obtained at 0.5 and 2 min and expressed as the uptake clearance (microliters per minute per 10^6 cells).

**Metabolism Study Using the Liver S9 Fraction**

It has been reported that pravastatin is metabolized by sulfotransferase in male rats (Kitazawa et al., 1993). Therefore, we used the liver S9 fraction as the enzyme source. The liver S9 fraction was prepared from four rats using standard procedures and stored at −80°C until use. The protein concentration was determined by the Lowry method, using bovine serum albumin as the standard. Pravastatin was incubated with a reaction mixture consisting of rat liver S9 fraction (final concentration, 8 mg/ml), NADPH-generating system (0.8 mM NADP^+), 8 mM glucose 6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, and 5 mM MgCl_2), and 3'-phosphoadenosine 5'-phosphosulfate (final concentrations, 0.5 and 5 mM for low and high pravastatin concentrations, respectively) in the presence of 100 mM phosphate buffer, pH 7.4. After preincubation at 37°C for 10 min, pravastatin (final concentration, 0.1–500 μM) was added to initiate the enzyme reaction. At the designated time, the reactions were terminated by mixing them with equal volumes of methanol containing R-122798, an analytical internal standard, followed by centrifugation at 15,000g for 10 min at 4°C. The supernatant was subjected to liquid chromatography/mass spectrometry (LC/MS) analysis. In studies with the human liver S9 fraction, the concentrations of pravastatin and 3'-phosphosulfate were 5 μM and 1 mM, respectively; other incubation conditions were the same as in the rat studies.

**LC/MS Analysis**

Liver, kidney, brain, lung, and skeletal muscle were added to 3 to 5 volumes of physiological saline (w/v) and homogenized. Tissue homogenates and plasma, bile, and urine samples were deproteinized with 2 volumes of methanol containing the internal standard (1 μg/ml R-122798) and centrifuged at 15,000g for 10 min at 4°C. High-concentration samples were diluted appropriately with blank matrix before deproteination. The supernatant was subjected to LC/MS analysis. The appropriate standard curves were prepared in the equivalent blank matrix and used for each analysis.

The LC/MS consisted of an Alliance HT 2695 separation module with an autosampler (Waters, Milford, MA) and a Micromass ZQ mass spectrometer with an electron ion spray interface (Waters). The optimum operating conditions used were as follows: electrospray probe (capillary) voltage, 3.2 kV; sample cone voltage, 20 V; and source temperature, 100°C. The spectrometer was operated at a drying desolvation gas flow rate of 350 l/h. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH− ions, m/z 423.3 for pravastatin and m/z 409.3 for the internal standard. The mobile phase used for high-performance liquid chromatography was acetonitrile/ammonium acetate buffer (10 mmol/L, pH 7.4), and the flow rate was 0.3 ml/min. Chromatographic separation was achieved on a C18 column (Inertsil ODS-3 column, 50 × 2.1 mm; particle size, 3 μm) (GL Sciences, Tokyo, Japan).

**Data Analysis of Metabolic Clearance in Liver S9**

The metabolic velocity was calculated from the slope of the natural log (concentration)-time plot. Because the Eadie-Hofstee plot showed curvature, the kinetic parameters were obtained using eq. 1:

\[
v = \frac{V_{\text{max}1} \times S}{K_{m1} + S} + \frac{V_{\text{max}2} \times S}{K_{m2} + S}
\]

where \(v\) is the initial velocity (picomoles per minute per milligram of protein), \(S\) is the substrate concentration (micromolar), \(V_{\text{max}1}\) and \(V_{\text{max}2}\) are the maximum velocities (picomoles per minute per milligram of protein), and \(K_{m1}\) and \(K_{m2}\) are the Michaelis constants (micromolar). Fitting was performed with the nonlinear least-squares method using the MULTIT program (Yamaoka et al., 1981).

The input data were weighted as the reciprocals of the observed values, and the Damping Gauss-Newton algorithm was used for fitting.

**Model Development**

The PBPK model was constructed to describe the pharmacokinetics of pravastatin in rats and humans (Fig. 1). The key features of this model are as follows: 1) Active uptake (PS_{int}) and passive diffusion clearances (PS_{dif}) on the sinusoidal membrane, and biliary clearance (PS_{bile}) on the canalicular membrane in the liver are incorporated. 2) The liver compartment consists of five units of extracellular and subcellular compartments, connected by blood flow in tandem, to fit the hepatic disposition to the “dispersion” model. Because the hepatic elimination of pravastatin in rats is blood flow limited, the dispersion model is the appropriate model for the hepatic elimination of such high-clearance drugs (Roberts and Rowland, 1986; Watahbo et al., 1997; Naritomi et al., 2001). The number of liver compartments was determined by comparing the hepatic availability \(F_{\text{h,n}}\) and \(F_{\text{h,s}}\) predicted using the dispersion model. \(F_{\text{h,n}}\) is the product of the availability in the liver compartments (eq. 2).

\[
F_{\text{h,n}} = \left( \frac{Q}{Q + f_s(CL_{\text{int,all}})^n} \right)^n
\]

where \(n\) represents the number of compartments. The integer number \(n\), which gave the \(F_{\text{h,n}}\) value closest to that in the dispersion model, was selected. 3) The brain and muscle, target tissues for the adverse effects of statins, were included. 4) Although urinary excretion is a minor elimination pathway in male rats, the kidney was included because the kidney/blood concentration ratio for pravastatin is high in male rats, probably because of the efficient uptake and/or reabsorption of pravastatin. The renal clearance of pravastatin in male rats was lower than the glomerular filtration rate corrected by the blood unbound fraction. In contrast, renal clearance must be taken into consideration in humans. Because this study
focused on hepatobiliary transport, renal elimination occurs from the systemic compartment. 5) The rapid equilibrium distribution of pravastatin between the blood and tissues other than the liver was assumed. 6) The initial distribution volume, estimated by fitting the plasma concentration time profiles of pravastatin in rats after the intravenous administration of 0.2 and 1 mg/kg to a two-compartment model, was used as the volume of the rapid equilibrium compartment including the blood compartment, and it was assumed that there is no interspecies difference in the initial distribution volume. The differential equations are shown in Appendix I, and all simulations were performed with SAAM II (SAAM Institute, Seattle, WA).

Estimation of Kinetic Parameters Used in the Simulation

**In Vitro Parameters (Rats and Humans).** The in vitro active uptake (PS\(_{\text{inf,vitro}}\)) and passive diffusion clearances (PS\(_{\text{dif,vitro}}\)) of pravastatin on the sinusoidal membrane were determined from the uptake studies using isolated hepatocytes. The parameters for rats were taken from previous reports (Yamazaki et al., 1993; Ishigami et al., 1995), and those for humans were determined in the present study. PS\(_{\text{inf,vitro}}\) and PS\(_{\text{dif,vitro}}\) were regarded as the saturable and nonsaturable components, respectively, in the uptake clearance into hepatocytes. A physiological scaling factor of 1.2 \(\times 10^9\) cells/g liver was used for scaling up to the organ level (Iwatsubo et al., 1997). The in vitro biliary clearance (CL\(_{\text{b,inf,vitro}}\)) of pravastatin was calculated from the ATP-dependent uptake clearance into the CMVs using eq. 3 (Niinuma et al., 1999):

\[
\text{PS}_{\text{b,inf,vitro}} = \frac{V_{\text{initial}} \times R}{E \times IO}
\]

where \(V_{\text{initial}}\) represents the velocity of the initial ATP-dependent uptake by CMVs corrected by medium concentration (6.08 \(\mu\)l/min/mg protein for rats and 1.90 \(\mu\)l/min/mg protein for humans), \(R\) represents the recovery of liver homogenate protein (174 mg homogenate protein/g liver for rats and 133 mg homogenate protein/g liver for humans), \(E\) represents the enrichment of the CMV fraction (70.4 for rats and 61.8 for humans), and IO represents the population of inside-out CMVs (0.347 for rats and 0.555 for humans).

**In Vivo Parameters (Rats).** The in vivo intrinsic biliary clearance (PS\(_{\text{b,vivo}}\)) at the canalicular membrane was calculated by dividing the biliary excretion rate by the hepatic unbound concentration at steady state (Yamazaki et al., 1996b, 1997). Systemic elimination other than biliary excretion was regarded as the hepatic unbound concentration. The plasma concentration data.

\[
\text{PS}_{\text{inf,vivo}} = \frac{\text{CL}_{\text{int,all}} \times \text{PS}_{\text{dif,vivo}} + \text{PS}_{\text{b,inf,vivo}} + \text{CL}_{\text{met,int,vivo}} \times \text{PS}_{\text{bile,vivo}} + \text{CL}_{\text{met,int,vivo}}}{\text{PS}_{\text{bile,vivo}} + \text{CL}_{\text{met,int,vivo}}} - \text{PS}_{\text{dif,vivo}}
\]

where \(\text{CL}_{\text{int,all}}\) represents the overall hepatic intrinsic clearance estimated from the hepatic availability using the dispersion model, with a dispersion number of 0.17, which was obtained by dividing the bioavailability by the fraction absorbed (Komai et al., 1992), assuming negligible metabolism in the small intestine. The average of the tissue/blood concentration ratios at 30, 60, and 90 min after the intravenous administration at 10 mg/kg pravastatin were used as the tissue/blood partition coefficient (\(K_p\)), assuming a pseudo-steady state (Table 2). Actually, the tissue/blood concentration ratios at 30, 60, and 90 min were similar (muscle, 0.28, 0.21, and 0.18; brain, 0.045, 0.029 and 0.034; kidney, 13, 14, and 15; lung, 0.76, 0.67, and 0.77 at 30, 60, and 90 min, respectively). The absorption rate constants were estimated by noncompartment analysis using the plasma concentration data.

**Results**

**In Vivo Pharmacokinetics of Pravastatin in Rats.** Figure 2 shows time profiles of the plasma concentration of pravastatin after its intravenous (0.2 mg/kg) and intraduodenal (20 mg/kg) administration and the cumulative amount of pravastatin excreted into the bile. The total blood clearance (CL\(_{\text{tot,B}}\)) was similar to the hepatic blood flow rate. The bioavailability of pravastatin after intraduodenal administration was calculated to be 0.0087 by comparing the AUC for pravastatin after intravenous and intraduodenal administration. Forty-six percent of the dose was recovered in the bile as the parent compound after intravenous administration, whereas the amount excreted into the urine was less than 4% of the dose. Even after intraduodenal administration, 33% was recovered in the bile.

The nonlinearity of the disposition of pravastatin was examined. The plasma concentrations and cumulative amounts excreted into the bile after its intravenous administration were determined at doses ranging from 0.2 to 200 mg/kg (Fig. 3). CL\(_{\text{tot,B}}\) was independent of the dose up to 50 mg/kg but decreased to 27 ml/min/kg at 200 mg/kg pravastatin. The cumulative biliary excretion increased slightly from 46 to 60% at doses above 0.2 mg/kg and was significantly delayed at 200 mg/kg.

**Hepatic Metabolism of Pravastatin in Rats.** The metabolism of pravastatin in the liver was examined using 59 fractions prepared from rat liver. It exhibited biphasic kinetics with high-affinity (\(K_{a1}\); 0.846 \(\pm\) 0.403 \(\mu\)M; \(V_{\text{max}1}\); 4.47 \(\pm\) 1.92 pmol/mg/min) and low-affinity (\(K_{a2}\); 80.3 \(\pm\) 12.6 \(\mu\)M; \(V_{\text{max}2}\); 240 \(\pm\) 16.2 pmol/mg/min) components (mean \(\pm\) S.D.).
The sum of the in vitro metabolic clearance for the high- and low-affinity components, corrected with the physiological scaling factor of 96.1 mg protein/g liver, was used as the in vitro metabolic clearance (CL_{met,int,vitro}), which was 0.793 ml/min/g liver (Table 1).

**Simulation of Concentration-Time Profiles of Pravastatin in Rats.** All parameters used in the simulation are summarized in Tables 1 and 2. The initial distribution volume was estimated to be 0.393 l/kg from the plasma concentration profile after the intravenous administration of 0.2 mg/kg pravastatin, which was used as the volume of the rapid equilibrium compartment in the model. Figures 2 and 3 show the simulated plasma concentrations and biliary excretion time profiles for pravastatin, together with the observed data after its intravenous and intraduodenal administration. To reproduce the in vivo pharmacokinetic profiles of pravastatin and 0.63 µl/min/10^6 cells at 1 µM pravastatin and 0.77 ± 0.63 µl/min/10^6 cells at 100 µM pravastatin (mean ± S.D.). Using the physiological scaling factor of 1.2 × 10^9 cells/g liver, PS_{inf,vitro,human} and PS_{dif,vitro,human} were calculated to be 0.448 and 0.0924 ml/min/g liver, respectively (Table 1). Unlike the rat liver S9 fraction, no metabolism of pravastatin was observed up to 180 min in the human liver S9 fractions purchased from three different vendors. Thus, the hepatic metabolism of pravastatin might be negligible in the human liver. The in vivo kinetic parameters for pravastatin in humans were predicted by multiplying the corresponding in vitro parameters obtained using human materials by the SF obtained from rat studies. For PS_{saturate} the saturable (ATP-dependent) biliary clearance in humans was predicted as described (Fig. 4). Although the model reasonably describes the experimental data, the simulated lines showed some deviation from the observed data at the terminal phase in Figs. 2 (left) and 5. This may be caused by the lack of a compartment corresponding to the organ, which is associated with the terminal phase of systemic pravastatin. Moreover, the simulation results of biliary excretion of pravastatin administered at 1 and 10 mg/kg showed some deviation from the observed data (Fig. 3, right). Because hepatic metabolism is saturated at these doses, this may be attributed to the deviation of the K_m value for metabolism.

### Prediction of Pharmacokinetics in Humans

The uptake clearance determined using eight lots of human cryopreserved hepatocytes was 4.5 ± 2.9 µl/min/10^6 cells at 1 µM pravastatin and 0.77 ± 0.63 µl/min/10^6 cells at 100 µM pravastatin (mean ± S.D.). Using the physiological scaling factor of 1.2 × 10^9 cells/g liver, PS_{inf,vitro,human} and PS_{dif,vitro,human} were calculated to be 0.448 and 0.0924 ml/min/g liver, respectively (Table 1). Unlike the rat liver S9 fraction, no metabolism of pravastatin was observed up to 180 min in the human liver S9 fractions purchased from three different vendors. Thus, the hepatic metabolism of pravastatin might be negligible in the human liver. The in vivo kinetic parameters for pravastatin in humans were predicted by multiplying the corresponding in vitro parameters obtained using human materials by the SF obtained from rat studies. For PS_{saturate} the saturable (ATP-dependent) biliary clearance in humans was predicted as described

### Table 1

<table>
<thead>
<tr>
<th>Rat</th>
<th>In Vitro</th>
<th>In Vivo</th>
<th>Human</th>
<th>In Vitro</th>
<th>In Vivo</th>
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<tr>
<td>PS_{saturate}</td>
<td>2.47</td>
<td>(32.8)</td>
<td>9.06</td>
<td>3.7</td>
<td>0.448</td>
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<td>PS_{inf}</td>
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<td>0.192</td>
<td>1</td>
<td>0.0924</td>
<td>0.0924</td>
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<tr>
<td>PS_{dif}</td>
<td>0.0433</td>
<td>0.906</td>
<td>0.234</td>
<td>21</td>
<td>0.00737</td>
</tr>
<tr>
<td>ATP-dependent</td>
<td>0.906</td>
<td>(92.3)</td>
<td>0.234</td>
<td>1.7</td>
<td>0.00737</td>
</tr>
<tr>
<td>Nonsaturable</td>
<td>0.793</td>
<td>1.33</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Predicted by multiplying the in vitro parameter by the SF.
* Yamazaki et al. (1993).
* Ishigami et al. (1995).
* Calculated using eq. 3 (Niinuma et al. (1999)).
* Yamazaki et al. (1997).
* Assumed that the SF for PS_{inf} is 1.
* Calculated using eq. 5.
* Calculated using eq. 4.
PBPK Model to Predict Pharmacokinetics of Pravastatin

Discussion

It is now well recognized that drug transporters play important roles in the processes of absorption, distribution, and excretion (Giacomini and Sugiyama, 2005; Shitara et al., 2006a). The purpose of this study was to construct a PBPK model to evaluate the concentration-time profiles for drugs in the plasma and peripheral organs in humans using physiological parameters, SFs, and drug-related parameters (unbound fraction and metabolic and membrane transport clearances extrapolated from in vitro experiments). The principle of the prediction was as follows. First, SFs were obtained by comparing in vitro and in vivo parameters in rats. Then, the in vitro human parameters were extrapolated in vivo using the SFs obtained in rats (Naritomi et al., 2001). Pravastatin was selected as the model compound because many studies have investigated the mechanisms involved in the drug disposition in rodents, and clinical data after intravenous and oral administration are available.

Consistent with a previous report (Yamazaki et al., 1996a), the hepatic elimination of pravastatin is blood flow limited. Considering that the maximum amount of intact pravastatin excreted into the bile was 50%, it is likely that pravastatin undergoes hepatic metabolism in rats because pravastatin is excreted negligibly in the urine. Incubating pravastatin with the rat liver S9 fractions caused a reduction in intact pravastatin under the same conditions as the in vitro liver S9 fractions. The kinetic parameters related to hepatic clearance (PSinf, PSdif, PSbile, and CLmet,int) were estimated from various in vivo experiments and were incorporated into the PBPK model. As a result, plasma concentration and biliary excretion-time profiles for pravastatin were successfully reproduced (Figs. 2–5). Moreover, nonlinear pharmacokinetics

Effect of Transporter Activity on Systemic and Target Exposure. Sensitivity analyses were performed to understand the effects of the changes in transporter activities on the time profiles for the plasma and liver (a target organ) concentrations of pravastatin in humans. The plasma and liver concentrations after the oral administration (40 mg) of pravastatin were simulated using the PBPK model constructed in this study, with varying hepatic transport activities over a range of 0.33 to 3.0 times the initial value. The simulated concentration-time profiles and the changes in the AUC are shown in Fig. 7 and Table 3, respectively. Changes in the active hepatic uptake ability affected the plasma concentration profiles dramatically but did not greatly affect the liver concentration profiles. On the contrary, changes in the ability of canalicular efflux altered the liver concentration of pravastatin markedly but had a small effect on the plasma concentration. Changes in the passive diffusion clearance hardly affect the plasma and the liver concentration profiles.
were also reproduced using the $K_m$ values for hepatic uptake, biliary excretion, and metabolic clearances (Fig. 3). The liver concentrations of pravastatin were similar to the observed data, even under nonlinear conditions (200 mg/kg) (Fig. 4). These results suggest that the PBPK model constructed in this study is appropriate for describing the pharmacokinetics of pravastatin in rats.

The kinetic parameters $PS_{\text{inh}}$, $PS_{\text{dif}}$, and $PS_{\text{bile}}$ were also determined in vitro using rat hepatocytes and CMVs to obtain the relevant SFs (Table 2). The corresponding parameters were also determined using human cryopreserved hepatocytes and CMVs. These parameters were extrapolated in vivo using the SFs determined in rats. Because there is no evidence that active transport mechanisms are involved in the sinusoidal efflux of pravastatin, the clearance corresponding to the nonsaturable component ($PS_{\text{dif}}$) of the uptake was used as the clearance for sinusoidal efflux. Unlike the rat liver S9 fractions, pravastatin was not metabolized in the human liver S9 fractions. Therefore, the metabolic clearance was set to zero in humans. Using the human parameters, simulated plasma concentration-time profiles of pravastatin after the intravenous and oral administration were fairly close to the observed data for humans (Fig. 6), showing that the predicted value was not far from the true value. It should be noted that the sinusoidal efflux clearance (passive diffusion clearance) was lower than the intrinsically biliary clearance with regard to the liver concentration, indicating that the hepatobiliary transport of pravastatin is likely uptake limited and that the hepatic intrinsic clearance can be approximated to $PS_{\text{inef}}$ (Shitara et al., 2006a). Therefore, even though the predictability of the absolute values for biliary and sinusoidal efflux clearance is low, the simulated results will be close to the observed data as far as the uptake clearance is correctly predicted. To validate the predictability of those clearances, the liver concentrations must be determined in humans, which should be possible with imaging technologies such as positron emission tomography, single-photon emission computed tomography, and magnetic resonance imaging. Ghibellini et al. (2007) recently developed a methodology for the real-time measurement of the biliary excretion profiles of drugs in humans using a gamma scintigraphy technique. Further efforts are required to use such in vivo imaging technologies to increase the predictability of these pharmacokinetic parameters.

To date, clinical studies have demonstrated that the genetic variations of OATP1B1 and drug-drug interactions involving OATP1B1 are associated with interindividual differences in the systemic exposure of pravastatin and other substrate drugs (Nishizato et al., 2003; Maeda et al., 2006; Niemi et al., 2006; Shitara and Sugiyama, 2006b). Because the pharmacological target of pravastatin is inside the cell, the liver exposure is a critical factor for its pharmacological activities. Based on the pharmacokinetic concepts, the AUC in the liver concentration is

![Fig. 5. Simulated and observed tissue concentration profiles for pravastatin in rats after intravenous administration at 10 mg/kg. Symbols and solid lines, experimentally observed and simulated values, respectively. Each point represents the mean ± S.E. (n = 3).](image-url)
governed only by the sequestration clearance from the liver as far as the renal elimination is negligible and is independent of the change in uptake clearance (eq. A4) (see Appendix II). When renal clearance makes a significant contribution, the changes in hepatic uptake activity can affect both the liver and the plasma AUC (Fig. 9; Appendix II). Actually, the renal elimination of pravastatin makes a significant contribution to the total body clearance (47% of the total body clearance) (Singhvi et al., 1990). Therefore, it is possible that the liver concentrations of pravastatin are affected to some extent also by the changes in the hepatic uptake activity. To support this concept, a simulation was performed with different uptake clearances (Fig. 7). Changes in the hepatic uptake clearance had a great impact on the plasma concentrations of pravastatin but less impact on the liver concentrations. In accordance, the effects of the genetic polymorphisms of OATP1B1 on the cholesterol-lowering effects of pravastatin will be small or absent at least at steady state (in other words, after relatively long-term treatment). The alteration of pharmacological effect of pravastatin with its chronic administration has not been observed in subjects with OATP1B1 polymorphisms although alteration of inhibitory effect of HMG CoA reductase activities in short-term treatments was reported (Takane et al., 2006; Kivisto and Niemi, 2007; Zhang et al., 2007). In contrast, changes in the intrinsic canalicular efflux activity should dramatically affect the liver concentration of pravastatin, whereas the plasma concentration is not affected as much by changes in the intrinsic biliary clearance (Fig. 7). Because the biliary excretion of pravastatin is mainly mediated by MRP2, the factors affecting MRP2 function, such as the use of MRP2 inhibitors or the genetic mutations causing Dubin-Johnson syndrome, will affect the pharmacological action of pravastatin. Furthermore, changes in the sinusoidal efflux clearance had only a slight impact on both the plasma and the liver concentrations. This is because, even under these conditions, the uptake process is still the rate-limiting process. Although the predictability of the sinusoidal efflux clearance remains unknown, changes within this range will not affect the simulated results.

One of the serious adverse effects of statins is myopathy (rhabdomyolysis). Because its target organ is the skeletal muscle, the systemic exposure should be the determinant factor of this adverse effect. The sensitivity analyses showed that the changes in the hepatic uptake clearance had a great impact on the systemic exposure of pravastatin, whereas those in the canalicular efflux had a minimal impact (Fig. 7). The results suggest that patients with an impaired OATP1B1 might be more susceptible to pravastatin-induced myopathy than those with normal one. Morimoto et al. (2004) reported that the frequency of the OATP1B1*15 haplotype was significantly higher in patients who experienced myopathy after receiving pravastatin or atorvastatin (which is also an OATP1B1 substrate) than in patients without myopathy, and a genome-wide study elucidated that the variants in OATP1B1 are strongly associated with an increased risk of simvastatin-induced myopathy (Link et al., 2008).

TABLE 3
Changes in the AUC (percentage of the control) for plasma and liver concentrations of pravastatin after its oral administration when the transporter function changes

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<thead>
<tr>
<th>Change in Clearance</th>
<th>PS_{inf} Plasma</th>
<th>PS_{inf} Liver</th>
<th>PS_{bile} Plasma</th>
<th>PS_{bile} Liver</th>
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<td>×1</td>
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<td>100</td>
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</tr>
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Fig. 7. Effects of changes in transporter activity on the time profiles of plasma and liver (target organ) concentrations of pravastatin in humans. Plasma and liver concentrations after oral administration (40 mg) were simulated using the PBPK model with varying hepatic transport activities over a 1/3- to 3-fold range of the initial values shown in Table 1. (— initial; –– ×1/3; ·······×3)
uptake clearance; PS_{dife}, passive diffusion clearance on the V_{met}, intrinsic metabolic clearance; F_{a}, fraction absorbed; K_{tin} concentration; Nomenclature

In the present study, a PBPK model, including transporter-mediated membrane transport processes, was constructed, which allows the prediction of the pharmacokinetics of pravastatin in humans. It also extends our understanding of the effects of changes in the transport processes on the pharmacological and adverse effects of drugs by simulating the exposure effects of changes in the systemic circulation and tissues to them. The present study suggests that changes in the OATP1B1 activities may have a small and a large impact on the therapeutic efficacy and side effect (myopathy) of pravastatin, respectively, whereas those in the MRP2 activities may have opposite impacts (i.e., a large and a small impact on the therapeutic efficacy and side effect).

Appendix I: Differential Mass Balance Equations for the PBPK Model

Nomenclature

General. Q, blood flow rate; V, tissue weight; C, pravastatin concentration; K_{p}, tissue/blood partition coefficient; f_{b}, blood unbound fraction; f_{T}, tissue unbound fraction; V_{m}, maximum transport velocity; K_{sat}, half-saturation concentration for transport; CL_{R}, renal clearance; PS_{inf}, intrinsic hepatic uptake clearance; PS_{diff}, passive diffusion clearance on the sinusoidal membrane; PS_{bile}, intrinsic biliary clearance; CL_{met}, intrinsic metabolic clearance; F_{a}, fraction absorbed; k_{a}, absorption rate constant.

Subscripts. B, blood; LU, lung; BR, brain; MU, muscle; R, kidney; GI, gastrointestinal tract; H, liver; HE, liver extracellular space; inf, influx; met, metabolism.

Model Equations

Hepatic uptake, biliary excretion, and metabolic clearances in humans were linear parameters.

Blood pool:

\[ V_{b}(dC_b/dt) = Q_{LU}(C_{LU}/K_{p,LU} - C_b) - CL_b C_b \]

Lung:

\[ V_{LU}(dC_{LU}/dt) = Q_{BR} C_{BR}/K_{p,BR} + Q_{MU} C_{MU}/K_{p,MU} + Q_R C_R/K_{p,R} + Q_H C_{HE} - Q_{LU} C_{LU}/K_{p,LU} \]

Brain, muscle, kidney:

\[ V_{i}(dC_i/dt) = Q_{i}(C_{B} - C_i/K_{p,i}) \]

Liver 1 to 5:

1) rat (\( V_{H}/5 \))(dC_{H}/dt) = (V_{m,inf}/5)f_{b} C_{HE}/(K_{m,inf} + f_{b} C_{HE}) + (PS_{diff}/5)f_{b} C_{HE} - (PS_{diff}/5)f_{b} C_{HE} - (V_{m,met}/5)f_{b} C_{HE} + (K_{m,bile} + f_{T} C_{Hi}) - (V_{m,met}/5)f_{b} C_{HE}/(K_{m,met} + f_{T} C_{Hi}) - (V_{m,met}/5)f_{b} C_{HE}/(K_{m,met} + f_{T} C_{Hi})

2) human (\( V_{H}/5 \))(dC_{H}/dt) = (PS_{inf}/5)f_{b} C_{HE} + (PS_{inf}/5)f_{b} C_{HE} - (PS_{inf}/5)f_{b} C_{HE} - (PS_{inf}/5)f_{b} C_{HE} - (CL_{met}/5)f_{b} C_{Hi}

Liver extracellular compartments 2 to 5:

1') rat (intravenous) (\( V_{H}/5 \))(dC_{H}/dt) = Q_{Lu}(C_{B} - C_{HE}) - (V_{m,inf}/5)f_{b} C_{HE}/(K_{m,inf} + f_{b} C_{HE}) - (PS_{diff}/5)f_{b} C_{HE} + (PS_{diff}/5)f_{b} C_{HE}

2) human (\( V_{H}/5 \))(dC_{H}/dt) = Q_{Lu}(C_{B} - C_{HE}) - (PS_{diff}/5)f_{b} C_{HE} - (PS_{diff}/5)f_{b} C_{HE} + (PS_{diff}/5)f_{b} C_{HE} + (PS_{diff}/5)f_{b} C_{HE} + k_{X_{GI}}

Liver extracellular compartments 1:

(1) rat (intravenous) (\( V_{H}/5 \))(dC_{H}/dt) = Q_{Lu}(C_{B} - C_{HE}) - (V_{m,inf}/5)f_{b} C_{HE}/(K_{m,inf} + f_{b} C_{HE}) - (PS_{diff}/5)f_{b} C_{HE} + (PS_{diff}/5)f_{b} C_{HE}

(1') rat (intravenous) (\( V_{H}/5 \))(dC_{H}/dt) = Q_{Lu}(C_{B} - C_{HE}) - (V_{m,inf}/5)f_{b} C_{HE}/(K_{m,inf} + f_{b} C_{HE}) - (PS_{diff}/5)f_{b} C_{HE} + (PS_{diff}/5)f_{b} C_{HE}

Bile or gastrointestinal tract:

(1) rat dX_{hi}/dt = \((V_{m,hi}/5)f_{b} C_{Hi}/(K_{m,hi} + f_{T} C_{Hi})\)

(1') rat (intravenous) dX_{gi}/dt = -(k_{a}/F_{a})X_{gi}

(2) human dX_{gi}/dt = \((PS_{inf}/5)f_{b} C_{Hi}/(K_{m,hi} + f_{T} C_{Hi})\) - (k_{a}/F_{a})X_{gi}

Appendix II: Effect of Renal Clearance on the Impact of the Change in the Uptake Clearance on the AUC of the Plasma and Liver

Q_{b} and CL_{b} represent the hepatic blood flow and renal clearance, respectively. PS_{inf}, PS_{diff}, and CL_{inf} are hepatic uptake, sinusoidal efflux, and intrinsic sequestration clearances, respectively. V and C represent volume and concentration, respectively. Subscripts b, i, and h represent blood, inlet, and liver, respectively. Mass balance differential equations for each compartment in the simple model shown in Fig. 8 are as follows:
Integrating these equations gives:

$$\frac{\text{Dose}}{f_h \cdot \text{AUC}_b} = \frac{\text{PS}_{\text{inf}} \cdot \text{CL}_{\text{int}}}{\text{PS}_{\text{eff}} + \text{CL}_{\text{int}}} \cdot \frac{\text{CL}_{\text{r}} + Q_h}{f_h \cdot \text{PS}_{\text{inf}}} \cdot C_b$$  (A1)

$$\frac{\text{Dose}}{f_h \cdot \text{AUC}_h} = \frac{\text{CL}_{\text{int}} + Q \cdot \text{PS}_{\text{eff}} + \text{CL}_{\text{int}}}{f_h \cdot \text{PS}_{\text{inf}}}$$  (A2)

where AUC$_b$ and AUC$_h$ represent the area under the concentration-time curve for the blood and liver, respectively. Substituting CL$_R$ = 0 yields:

$$\frac{\text{Dose}}{f_h \cdot \text{AUC}_b} = \frac{\text{PS}_{\text{inf}} \cdot \text{CL}_{\text{int}}}{\text{PS}_{\text{eff}} + \text{CL}_{\text{int}}}$$  (A3)

$$\frac{\text{Dose}}{f_h \cdot \text{AUC}_h} = \frac{\text{CL}_{\text{int}}}{f_h \cdot \text{PS}_{\text{inf}} \cdot Q} \cdot \frac{Q_r}{Q_h} = 9$$  (A4)

Equations A3 and A4 indicate that AUC$_b$ depends only on CL$_{\text{int}}$ when the renal clearance is negligible. In contrast, AUC$_h$ is inversely proportional to PS$_{\text{inf}}$. If the renal clearance is maximal, that is, the renal blood flow ($Q_r$), eq. A2 can be converted to:

$$\frac{\text{Dose}}{f_h \cdot \text{AUC}_h} = \frac{\text{CL}_{\text{int}} + Q \cdot \text{PS}_{\text{eff}} + \text{CL}_{\text{int}}}{f_h \cdot \text{PS}_{\text{inf}}} \cdot \frac{\text{CL}_{\text{r}} + Q_h}{f_h \cdot \text{PS}_{\text{inf}}}$$  (A5)

where

When the hepatic uptake is the rate-limiting process, so CL$_{\text{int}} >>$ PS$_{\text{eff}}$, eq. A5 can be converted to:

$$R = \frac{\text{Dose}}{f_h \cdot \text{AUC}_h} = \frac{\text{CL}_{\text{int}}}{f_h \cdot \text{PS}_{\text{inf}}} \left( 1 + Q \cdot \frac{\text{CL}_{\text{int}}}{f_h \cdot \text{PS}_{\text{inf}}} \right)$$  (A6)

In accordance, the $R$ value can be higher than CL$_{\text{int}}$ by up to $Q \times$ CL$_{\text{int}}/(f_h \times \text{PS}_{\text{inf}})$. Figure 9 shows the effects of renal
clearance on the impact of the changes in hepatic uptake and intrinsic sequestration clearance on the AUC of pravastatin in the plasma and liver. A simulation was performed using eqs. A1 and A2 and the parameters shown in Tables 1 and 2, when the renal clearance was 0, 4 (one quarter of the renal blood flow), and 16 (the renal blood flow) ml/min/kg b.w.

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References


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