Title:

Organic anion transporting polypeptides (OATPs)-mediated drug-drug interaction study between rosvastatin and cyclosporine A in chimeric mice with humanized liver.

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Running title: OATPs-mediated DDI in chimeric mice with humanized liver.

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Abbreviations: AUC: area under the blood concentration-time curve, AUCR: AUC ratio, BCRP: Breast cancer resistance protein, CYP: cytochrome P450, DDI: drug-drug
Abstract

The influence of transporters on the pharmacokinetics of drugs is being increasingly recognized, and DDIs via transporters may be a risk factor for adverse events. Cyclosporine A, a strong OATP-inhibitor, has been reported to increase the systemic exposure of rosuvastatin, an OATP-substrate, by 7.1-fold in clinical studies. PXB-mice are chimeric mice with humanized livers that are highly repopulated with human hepatocytes, and have been widely used for drug discovery in DMPK studies. In the present study, we examined in vivo and in vitro DDIs between rosuvastatin and cyclosporine A in PXB-mice and fresh human hepatocytes (PXB-cells) obtained from PXB-mice. We initially investigated the active transport of rosuvastatin into PXB-cells, and found concentration-dependent uptake with $K_m$ of 4.0 μmol/L and $V_{max}$ of 4.63 pmol/min/10^6 cells. Cyclosporine A inhibited the uptake of rosuvastatin with $IC_{50}$ of 0.21 μmol/L. We then examined in vivo DDIs, and the exposure of orally administered rosuvastatin increased by 3.3- and 11-fold in PXB-mice pretreated with 10 and 50 mg/kg of cyclosporine A, whereas by 2.5- and 6.2-fold when rosuvastatin was administered intravenously, that were conducted for considering gastrointestinal DDIs. The liver-to-blood concentration ratio of rosuvastatin was dose-dependently decreased by a pretreatment with cyclosporine A in PXB-mice and SCID mice. Observed DDIs in

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vivo were considered to be reasonable based on the estimated concentrations of cyclosporine A at the inlet to the liver and in the liver tissues of both mice. In conclusion, our results indicate that PXB-mice might be a useful tool for predicting human OATPs-mediated DDIs in drug discovery, and also its limitation due to the differences of gastrointestinal condition from human should be considered.
Introduction

The influence of transporters on the pharmacokinetics of drug is being increasingly recognized. Drug-drug interactions (DDIs) mediated by transporters may be a risk factor for adverse events, and recent studies demonstrated increased systemic exposure of victims by combination dosing with inhibitors of various transporters. Therefore, guidelines in the U.S., Europe, and Japan now request safety assessments of DDIs in drug development by large-scale clinical trials (CDER, 2012; CHMP, 2012; PMDA, 2014).

Organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3 are mainly expressed in the sinusoidal membrane of human hepatocytes, and solute carrier (SLC) transporters playing an important role in the hepatic uptake of drugs. Clinical drug interactions via hepatic OATPs are particularly relevant to statins (Neuvonen et al., 2006; Giacomini et al., 2010). The systemic exposure of rosuvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase inhibitor, that is widely used in the treatment of hypercholesterolemia, was significantly increased by concomitant dosing with cyclosporine A (Simonson et al, 2004), and commonly lower dosage of rosuvastatin is therefore required when administered with cyclosporine A in clinical. Rosuvastatin has low passive membrane permeability, and its systemic
clearance is mostly mediated by hepatic uptake and biliary efflux transporters (Martin et al., 2003a, 2003b). Multiple hepatic uptake transporters, e.g., OATP1B1, OATP1B3 and OATP2B1, and efflux transporters, e.g., breast cancer resistance protein (BCRP) and multi-drug resistance protein 2 (MRP2), are involved in its transport (Ho et al., 2006; Huang et al., 2006; Ellis et al., 2013). However, hepatic uptake of rosuvastatin via OATPs significantly influences its pharmacokinetics rather than biliary efflux of rosuvastatin via other transporters, because the uptake process is the rate-limiting step in its disposition from the systemic circulation (Shitara and Sugiyama, 2006; Maeda et al., 2011; Shitara et al., 2013).

The *in vivo* DDI studies in experimental animals have been conducted to predict DDIs in human (Lau et al., 2006; Imaoka et al., 2013; Shen et al., 2013; Takahashi et al., 2013; Chu et al., 2015), but the predictability of traditional preclinical animal models may be limited by species differences (Giacomini et al., 2010; Chu et al., 2015). Orthologous human *OATP* genes have not been identified for many rat/mouse *Oatp* genes, indicating the absence of a strict one-to-one relationship between rodent *Oatps* and human *OATPs* (Hagenbuch and Meier, 2003). Knockout models lacking murine oatp isoforms and OATP-humanized transgenic mice have been developed in order to estimate the pharmacokinetics of drugs and interactions with concomitants via human
OATPs (van de Steeg et al., 2013; Zimmerman et al., 2013; Higgins et al., 2014; Salphati et al., 2014). However, the drug disposition of some drugs may be affected by other murine transporters (Iusuf et al., 2013; Chang et al., 2014; Salphati et al., 2014).

PXB-mice are chimeric mice with humanized livers that are highly repopulated with human hepatocytes (Tateno et al., 2004, 2015). The expression levels (both mRNA and protein) of metabolic enzymes and transporters in human hepatocytes of PXB-mice were previously reported to be similar to those in humans (Tateno et al., 2004; Nishimura et al., 2005; Okumura et al, 2007; Ohtsuki et al., 2014), and PXB-mice have been characterized in detail and widely used in drug discovery to predict human-specific metabolite identification (Samuelsson et al., 2012; Bateman et al., 2014), induction and inhibition of drug metabolic enzymes (Katoh et al., 2005, 2007; Hasegawa et al., 2012; Kakuni et al., 2014), human pharmacokinetics (Sanoh et al., 2012, 2015; Miyamoto et al., 2017), and secretion into bile (Okumura et al., 2007).

In the present study, we examined the utility of PXB-mice to predict human OATP-mediated DDIs between rosuvastatin, a typical OATP-substrate, and cyclosporine A, a strong OATP-inhibitor. Rosuvastatin is more hydrophilic than other statins and is mainly excreted into bile in its unchanged form with negligible metabolism (Martin et al, 2003a, 2003b); however, two metabolites, the lactone form (a
metabolite via uridine diphosphate glucuronosyltransferase 1A, UGT1A) and N-desmethyl form (a metabolite via cytochrome P450 2C, CYP2C) have been identified (Prueksaritanont et al., 2002; Martin et al., 2003b). In the present study, these metabolites were also monitored in order to identify the replacement of human livers in PXB-mice as well as the inhibitory effects of cyclosporine A on the metabolism of rosvastatin.

Materials and Methods

Chemicals

Rosuvastatin and cyclosporine A were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). \( \text{d6-rosuvastatin, rosvastatin lactone, N-desmethyl rosvastatin,} \) and cyclosporine AM1 were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). All other reagents and solvents were commercial products of the highest available grade or analytical grade.

Animals

The present study was approved by the Ethics Committees of PhoenixBio Co., Ltd. and Toray Industries, Inc., and animal experiments were conducted according to the
Guidelines for Animal Experiments, PhoenixBio Co., Ltd. and Toray Industries, Inc. In order to generate chimeric mice with humanized livers, cryopreserved human hepatocytes (a 2-years-old Hispanic female), which had been donated with informed consent, were purchased from BD Biosciences (Woburn, MA, USA). Chimeric mice with humanized livers (PXB-mice®) were generated using a previously reported method (Tateno et al., 2015). Briefly, $1.0 \times 10^6$ human hepatocytes were injected into the inferior splenic pole of 2- to 4-week-old male cDNA-uPA$^{\text{wild/+}}$/SCID mice. Mice were used in experiments between 17 to 19 weeks of age and with a body weight of 16.0 to 23.4 g. The replacement index or extent of repopulation of the chimeric mouse liver with human hepatocytes was 92 to 99%, as assessed by the measurement of human albumin levels in blood (Tateno et al., 2015). Native SCID mice were used as controls. SCID mice were obtained from Charles River Laboratories Japan Inc. (Yokohama, Japan).

**Human Hepatocytes Isolated from Chimeric Mice, PXB-cells**

Approximately 1.5 to $1.7 \times 10^8$ human hepatocytes (PXB-cells®) were obtained from a PXB-mouse (repopulation ratio: 89 to 91%) using the collagenase perfusion method (Yamasaki et al., 2010). Fresh human hepatocytes attached to 24-well cell plates confluenctly at a density of $2.1 \times 10^5$ cells/cm$^2$ ($4.0 \times 10^5$ cells/well), and were
maintained for 2 to 4 weeks with d-HCGM medium consisting of Dulbecco's modified Eagle's medium (Yamasaki C et al., 2006).

**In Vitro Time- and Concentration-dependent Uptake of Rosuvastatin in PXB-cells**

Prior to the assay, cells were rinsed twice with pre-warmed PBS (37°C) and equilibrated in HBSS for 2 minutes. Uptake studies were initiated by removing the equilibrating buffer and adding 0.50 mL of HBSS containing 0.1 to 30 μmol/L of rosuvastatin. Hepatocytes were then incubated at 37°C in a humidified 5% CO₂ atmosphere. At each of the designed time points, the incubation was terminated by removing the transport buffer, and cells were immediately washed with ice-cold PBS three times. After adding 0.5 mL of acetonitrile containing 0.1% formic acid to each well, cells were scraped and recovered to another tube using a pipette. The concentrations of rosuvastatin were measured by LC-MS/MS. In order to assess the passive uptake of rosuvastatin in the hepatocytes, the same experiment was conducted on ice.

**In Vitro DDI for the Uptake of Rosuvastatin with Cyclosporine A in PXB-cells**

The *in vitro* inhibitory effects of cyclosporine A on the uptake of rosuvastatin in PXB-cells were examined using a similar method as the uptake studies on rosuvastatin
described above. Uptake studies were initiated by removing the equilibrating buffer and adding 0.50 mL of HBSS containing between 0.01 and 3 μmol/L of cyclosporine A, and 1 μmol/L of rosvastatin. Cells were incubated at 37°C for 10 minutes.

**In Vivo DDI for the Pharmacokinetics of Rosuvastatin with Cyclosporine A in PXB-mice**

Cyclosporine A was dissolved and formed a clean yellow-colored solution with ethanol and olive oil (1:9, v/v). Cyclosporine A or vehicle (5 mL/kg) was administered orally to mice at a dose of 10 or 50 mg/kg 4 hours before the oral administration of rosvastatin. Rosuvastatin was dissolved in saline containing 1% DMSO for its intravenous administration, and suspended in 0.3% HPMC for its oral administration. Rosuvastatin (5 mL/kg) was administered intravenously to mice at a dose of 1 mg/kg, or orally at a dose of 2 mg/kg. In the pharmacokinetics analysis, thirty microliters of blood was taken at the designated time points, 0.25, 0.5, 1, 2, and 4-hour post-dose (after oral administration) and 0.083, 0.25, 1, 2, and 4-hour post-dose (after intravenous administration), from the orbital vein, mixed with an equal volume of 20 mmol/L of ice-cold sodium acetate buffer (pH 4.0), and immediately placed on ice to prevent the degradation of the lactone form of rosvastatin. At termination (4 hours after the oral...
administration of rosuvastatin), all animals were euthanized by bleeding via the heart under isoflurane anesthesia, and each liver was collected to measure the tissue concentrations of rosuvastatin and cyclosporine A in the liver. Collected livers were rinsed, weighed, and homogenized in a 4-fold volume of 20 mmol/L of ice-cold sodium acetate buffer (pH 4.0) by the homogenizer, Shake Master Neo (BMS-M10N21, Biomedical Sciences, Tokyo, Japan). Treated blood and liver homogenates were stored at -20°C or lower for later bioanalyses.

**Fraction Unbound of Cyclosporine A in Blood**

Because we could not obtain fresh blood of PXB-mice due to transport issue from the supplier to us, we used ICR mice blood instead. Heparinized fresh blood was collected from ICR mice (11 years old, male, Charles River Japan). 10 μL of cyclosporine A (10, 100 μmol/L) was added to 490 μL of the fresh blood (final concentration: 0.2, 2 μmol/L), mixed and incubated for 30 minutes in a water bath (NTS-4000BM, Tokyo Rikakikai) set at 37°C. Samples were centrifuged at 1,800 × g for 10 minutes (S700RF, Kubota). The obtained plasma was analyzed by LC-MS/MS described below. Blood to plasma concentration ratio (Rb) was calculated from the equation: 

\[ \frac{C_{\text{blood}}}{C_{\text{plasma}}} \]

Five microliter of cyclosporine A (100, 1000 μmol/L) was added to 2,495 μL of heparinized
plasma of PXB-mice (final concentration: 0.2, 2 μmol/L), mixed and incubated for 10 minutes in the water bath set at 37°C. Samples were centrifuged at 608,000 × g for 120 minutes at 25°C (CS120GXL, Hitachi). The clear middle layer of obtained plasma, protein-free fraction, was collected and analyzed by LC-MS/MS described below.

Fraction unbound in blood $f_{ub}$ in PXB-mice was calculated in the equation: $1 - \frac{(C_{plasma} - C_{unbound})}{C_{unbound}} / Rb.$

**Bioanalysis by Liquid Chromatography and Tandem Mass spectrometry**

Analytes in pre-treated blood and liver homogenates were extracted by adding acetonitrile containing 0.1% formic acid. After vortexing the extraction mixture and centrifuging, each supernatant was filtrated, diluted with 0.1% acetic acid, and analytes were quantified by LC-MS/MS with the liquid chromatography, Nexera (Shimadzu Corporation, Kyoto, Japan) and the mass spectrometer, QTRAP5500 (SCIEX, Concord, Ontario, Canada). Chromatographic separation was performed with CAPCELLPAK C18 MGIII, 50 × 2.0 mm, 5 μm. (Shiseido Co., Ltd., Tokyo, Japan). Mobile phase A consisted of distillated water containing 0.1% formic acid, while mobile phase B consisted of acetonitrile. The initial % of B was 10%. A linear gradient was applied from 10 to 70%B over 3 minutes, 70 to 95%B over 2 minutes, maintained at 95%B for
1 minute, and decreased to 10% B for equilibration over 3 minutes. The total flow rate of the mobile phases was 0.4 mL/min. The mass spectrometer spray voltage was set at 5.5 kV, while the probe temperature was 600°C with positive ion polarity. Rosuvastatin, its lactone form, its N-desmethyl form, cyclosporine A, its hydroxy form AM1, and \textit{d6-rosuvastatin}, which was the internal standard, were measured by multiple reaction monitoring. Each precursor to product ion transitions was \textit{m/z} 482 to 258 for rosuvastatin, \textit{m/z} 464 to 270 for rosuvastatin lactone, \textit{m/z} 468 to 258 for N-desmethyl rosvustatin, \textit{m/z} 1203 to 425 for cyclosporine A, \textit{m/z} 1219 to 425 for cyclosporine AM1 and \textit{m/z} 488 to 264 for \textit{d6-rosuvastatin}.

**Data Processing**

**In Vitro Transport Activity**

The \textit{in vitro} uptake rates of rosuvastatin in PXB-cells were calculated using the below equation 1:

\[
\text{Uptake Rate (pmol/min/well)} = \frac{\text{Uptake Amount of Rosuvastatin in PXB-cells per Well (pmol/well)}}{\text{Incubation Time (min)}} \quad (1)
\]

A kinetic analysis of the transporter-mediated uptake of rosuvastatin in PXB-cells was performed with the extended Michaelis-Menten equation, and \(K_m\) and \(V_{max}\) were
calculated using the following equation 2:

\[
Uptake\ Rate = \frac{V_{max} \times S}{K_m + S} + P_{dif} \times S \tag{2}
\]

where \(S\) is the substrate concentration of rosuvastatin in the incubation buffer (\(\mu\text{mol/L}\)), \(K_m\) is the Michaelis-Menten constant (\(\mu\text{mol/L}\)), and \(V_{max}\) is the maximum uptake rate (pmol/min/\(10^6\) cells). \(P_{dif}\) represents non-saturable uptake clearance via passive diffusion (\(\mu\text{L/min/}10^6\) cells). Fitting was performed by a non-linear least-squares regression method using Phoenix WinNonlin ver. 6.2 (Certara Inc., Prinston, NJ, USA).

In addition, the uptake rate of rosuvastatin in cells (as the Y-axis) was plotted against the common logarithm of the concentration of cyclosporine A (as the X-axis), and a regression analysis was performed using the equation “log (inhibitor) vs. response variable slope (four parameters)” of GraphPad Prism ver. 5.0 (GraphPad Software Inc., San Diego, CA, USA) to obtain IC\(_{50}\). The equation is 4-parameter logistic regression shown below.

\[
Y = A(\infty) + \frac{A(0) - A(\infty)}{1 + 10^{((\log I_{50} - X) \times \text{Hillslope})}} \tag{3}
\]

Where \(A(\infty)\) is the maximum inhibitory effect observed by an inhibitor, and \(A(0)\) is the minimum inhibitory effect in the absence of an inhibitor, which should be the maximum uptake rate of rosuvastatin in the study.
Pharmacokinetics Analysis

Pharmacokinetic parameters were calculated by a non-compartmental method using Phoenix WinNonlin ver 6.2.

Estimation of the Maximum Protein-unbound Concentration of Cyclosporine A at the Inlet to the Liver, and Observed Concentrations of Cyclosporine A in Blood and Liver Tissue

In order to assess the relationship between inhibitor concentrations and inhibitory effects against each transporter, we estimated the maximum unbound concentration of cyclosporine A at the inlet to the liver as an effective concentration of the inhibitor, and compared it with the reported IC$_{50}$s as the inhibitory effect against each transporter. The maximum unbound concentration of cyclosporine A at the inlet to the liver was calculated based on its systemic concentration in blood using the below equation 4:

\[ I_{in, max, u} = f_{u,b} \times (I_{max, b} + F_a F_g \times k_a \times Dose/Q_h) \]  \hspace{1cm} (4)

where $f_{u,b}$ is the unbound fraction of the inhibitor, cyclosporine A, in blood, $I_{max,b}$ is the
maximum circulating blood concentration of the inhibitor, which is equal to $C_{\text{max}}$, $k_a$ is the absorption rate constant of the inhibitor, $F_a$ is the fraction of the inhibitor dose absorbed, $F_g$ is the fraction of the absorbed inhibitor dose escaping gut wall extraction, Dose is the inhibitor dose, and $Q_h$ is the hepatic blood flow rate in mice. In addition, the concentrations of cyclosporine A in blood at the same sampling points as rosuvastatin and the liver at the terminal point, eight hours after the oral administration of cyclosporine A (at the same time as four hours after the oral administration of rosuvastatin) were measured in PXB-mice and SCID mice.

Statistical Analysis

The student’s t-test ($p < 0.05$) was applied to assess the statistical significance of each parameter and value in the in vivo DDI study between rosuvastatin and cyclosporine A.

Results

In Vitro Time- and Concentration-dependent Uptake of Rosuvastatin in PXB-cells

Rosuvastatin was incubated with PXB-cells at a concentration of 1 μmol/L and temperature of 37°C, and uptake into cells was observed in a time-dependent manner almost linearly up to 10 minutes. The transport rate was 1.38 pmol/min/well (Fig. 1a).
On the other hand, the passive uptake of rosuvastatin into cells was assessed with an incubation on ice, and the transport rate was 0.0693 pmol/min/well, which was 20-fold lower than that at 37°C (Fig. 1a). Furthermore, in order to assess the concentration-dependent kinetics of the active uptake of rosuvastatin in PXB-cells, rosuvastatin was incubated at 37°C and on ice at different concentrations between 0.1 and 30 μmol/L. The active uptake amount of rosuvastatin was calculated by subtracting each value obtained on ice from the corresponding value at 37°C. The Michaelis-Menten equation showed that \( K_m \) and \( V_{max} \) were 4.0 μmol/L and 4.63 pmol/min/10^6 cells (1.85 pmol/min/well), respectively (Fig. 1b).

**In Vitro DDI for the Uptake of Rosuvastatin with Cyclosporine A in PXB-cells**

The inhibitory effects of cyclosporine A on the uptake of rosuvastatin in PXB-cells were examined. The uptake of rosuvastatin was inhibited in a concentration-dependent manner of cyclosporine A, and IC\(_{50}\) was 0.21 μmol/L (Fig. 1c).

**In Vivo DDI between Rosuvastatin and Cyclosporine A in PXB-mice**

In order to investigate the effects of cyclosporine A on the pharmacokinetics of rosuvastatin, systemic concentrations of rosuvastatin were measured with or without
cyclosporine A in PXB-mice and SCID mice as the control. When only rosvastatin was administered orally at a dose of 2 mg/kg to PXB-mice, the maximum concentration in blood ($C_{\text{max}}$) and area under the concentration-time curve in blood from initial to four hours after its administration ($AUC_{0-4h}$) were 46.7 ng/mL and 105 ng·h/mL, respectively (Fig. 2a, Table 1). However, when cyclosporine A was administered orally at doses of 10 and 50 mg/kg four hours before the administration of rosvastatin, the systemic exposure, AUCs, of the orally administered rosvastatin increased significantly by 3.3- and 11-fold, respectively (Fig. 2a, Table 1). On the other hand, rosvastatin was also administered to SCID mice in order to assess the control pharmacokinetic profile of rosvastatin in mice, and $C_{\text{max}}$ and $AUC_{0-4h}$ were 3.31 ng/mL and 5.14 ng·h/mL, respectively, after the oral administration of rosvastatin at a dose of 2 mg/kg without cyclosporine A (Fig. 2b, Table 1). These results showed that the systemic exposure of rosvastatin in SCID mice was approximately 20-fold lower than that in PXB-mice. Furthermore, the effects of cyclosporine A on the pharmacokinetics of rosvastatin in SCID mice were investigated, and the AUCs of rosvastatin increased by 13-fold with co-administration orally of 10 mg/kg of cyclosporine A four hours before the administration of rosvastatin, and further increased by 78-fold with 50 mg/kg of cyclosporine A (Fig. 2b, Table 1). In order to assess the uptake of rosvastatin into cells
in liver tissue and the inhibitory effects of cyclosporine A on uptake in PXB-mice and SCID mice, liver concentrations of rosuvastatin four hours after its oral administration with or without cyclosporine A were measured. The $K_{p,\text{ liver}}$ value was calculated using the concentration ratio of rosuvastatin in the liver over that in blood. Its $K_{p,\text{ liver}}$ value was higher in SCID mice (336) than in PXB-mice (44) and decreased with the administration of cyclosporine A in a dose-dependent manner in PXB-mice and SCID mice (Fig. 3).

In the present study, the major metabolites of rosuvastatin reported in humans, which are the lactone and N-desmethyl forms, were also measured in blood and liver tissue; however, the lactone form of rosuvastatin was not detected in the blood of PXB-mice or SCID mice (lower than 3 ng/mL), but was present in liver tissues. The concentration ratios of the lactone form against rosuvastatin in the liver exhibited a strain difference: 37 to 50% in PXB-mice, but only 1% in SCID mice. On the other hand, the N-desmethyl form was detected in PXB-mice only. The exposure ratios of the N-desmethyl form against rosuvastatin were 4 to 6% in blood, and 2% in liver tissue. Metabolism to these two metabolites of rosuvastatin was not affected by the co-administration of cyclosporine A in PXB-mice and SCID mice.

As described above, blood concentrations of rosuvastatin administered orally were
lower in SCID mice than in PXB-mice, and thus an intravenous administration study of rosuvastatin was conducted in order to assess the systemic clearance of rosuvastatin in SCID mice and PXB-mice. Systemic exposure, AUC$_{0-4h}$, after an intravenous dose of 1 mg/kg of rosuvastatin was higher in PXB-mice (280 ng·h/mL) than in SCID mice (26.2 ng·h/mL), and similar results were obtained after the oral administration of rosuvastatin. The total systemic clearance of rosuvastatin was 3.82 L/h/kg in PXB-mice, and 38.1 L/h/kg in SCID mice (Table 1).

**Estimation of the Maximum Protein-unbound Concentration of Cyclosporine A at the Inlet to the Liver, and Observed Concentrations of Cyclosporine A in Blood and Liver Tissue**

The systemic exposure of cyclosporine A was measured from four to eight hours after its oral administration at the same sampling time points as those for rosuvastatin. The results obtained revealed that the systemic exposure of cyclosporine A increased in a dose-dependent manner at doses of 10 mg/kg and 50 mg/kg of cyclosporine A in PXB-mice and SCID mice (Table 3). Based on the systemic exposure of cyclosporine A, the maximum protein-unbound concentration of cyclosporine A at the inlet to the liver, I$_{in, max, u}$, was estimated in order to clarify the relationship between cyclosporine A
concentrations and its inhibitory effects on each transporter in the liver. Several pharmacokinetics and physiological parameters were referred from previous studies to calculate $I_{in, max, u}$ in the present study: $f_a, b$ were 10% in human (Yoshida et al., 2012) and 10% in mice obtained in the study (Supplemental Table 2). $Q_h$ was 1.8 mL/min/0.02 kg (Davies and Morris, 1993). $F_{a}F_{g}$ was regarded as 0.3 to 1.0 (Yoshida et al., 2012; Kato, 2008; Kadono et al., 2014) and $k_a$ was regarded as 0.1 min$^{-1}$ to minimize the risk of the false-negative prediction of DDI (Yoshida et al, 2012; Izumi et al. 2015; CDER, 2012; CHMP, 2012; PMDA, 2014). We consequently estimated that the $I_{in, max, u}$ of cyclosporine A in PXB-mice were 0.29 to 0.94 and 1.4 to 4.7 μmol/L at doses of 10 and 50 mg/kg of cyclosporine A, respectively, and were 0.36 to 0.96 and 1.5 to 4.8 μmol/L at doses of 10 and 50 mg/kg, respectively in SCID mice (Table 2). In the present study, the concentrations of cyclosporine A in the blood at the same sampling points as rosuvastatin and in the liver eight hours after the oral administration of cyclosporine A (the same time as four hours after the oral administration of rosuvastatin) were measured in PXB-mice and SCID mice in order to assess not only its exposure at the inlet to the liver, but also systemic and liver exposures. Liver concentrations were 1.07 and 5.89 μmol/kg tissue at doses of 10 and 50 mg/kg, respectively, in PXB-mice, and were 4.95 and 30.0 μmol/kg tissue at doses of 10 and 50 mg/kg, respectively, in SCID
mice (Table 3). The reported $I_{\text{in, max, u}}$ of cyclosporine A was 1.2 to 1.3 $\mu$mol/L (Yoshida et al., 2012; Izumi et al., 2015) when a 7.1-fold increase in the systemic exposure of rosvastatin was observed in a clinical study (Simonson et al., 2004), and the IC$_{50}$s of cyclosporine A to OATP1B1, OATP1B3, MRP2, BCRP, and MDR1 were 0.15, 0.68, 9.3, 1.5, and 1.7 $\mu$mol/L, respectively (Table 3) (Yoshida et al., 2012).

**Discussion**

In the present study, we demonstrated *in vitro* and *in vivo* DDIs between rosvastatin, a substrate of OATPs, and cyclosporine A, a strong inhibitor of OATPs, in PXB-cells and PXB-mice. Okumura et al. investigated the gene expression of human transporters in human hepatocytes of PXB-mice, and reported that the expression of OATP1B1/1B3 was strong, whereas that of oatp1b2 was weak (Okumura et al., 2007). They also reported that the mRNA expression levels of hepatic transporters in the PXB-mice and donor hepatocytes, and the levels of OATP1B1/1B3 in PXB-mice were almost same, 0.58 to 1.58 higher than, as those in donor hepatocytes, which would indicate that the intestinal-liver axes does not affect expressions of OATPs in the mice model rather than the other (Chow et al., 2017). Ohtsuki et al. also conducted the protein qualification of transporters using a LC-MS/MS technique, and showed similar expression levels for
human transporters between human hepatocytes of PXB-mice and 17 human liver biopsies, no significant deference of the protein expression of OATP1B1/1B3 between three different donors (2, 5, and 10 years old) of hepatocytes of PXB-mice (Ohtsuki et al., 2014). Unfortunately, we do not have any genotype information of the SLCO1B1 in PXB-mice we used in the study. PXB-cells are fresh human hepatocytes isolated from PXB-mice, and have been used in in vitro DMPK studies (Yamasaki et al., 2010; Kakuni et al., 2014).

We evaluated the transporting activity of rosuvastatin as a probe for OATPs, and in vitro DDI between rosuvastatin and cyclosporine A in PXB-cells, and the results obtained showed time- and temperature-dependent uptake. The transport of rosuvastatin at 1 μmol/L into PXB-cells was strongly controlled by active uptake via transporters, and the contribution ratio of active uptake against total uptake was calculated to be 95%, similar to the reported values of 84 to 93% (Bi et al., 2012). The active transport of rosuvastatin occurred in a concentration-dependent manner with $K_m$ of 4.0 μmol/L and $V_{max}$ of 4.63 pmol/min/10^6 cells, whereas reported $K_m$ were 10 to 11 μmol/L and $V_{max}$ were 16.3 to 114 pmol/min/10^6 cells in human hepatocytes (Menochet et al., 2012; Shen et al., 2013). In the study, we performed the conventional approach of parallel experiments at 37°C and 4°C based on an extended Michaelis-Menten equation, which contains passive
clearance but does not consider bidirectional nature of the passive process and intracellular binding (Zamek-Gliszczynski et al., 2013). Those processes would be carefully considered specially to estimate precise quantitative relationship between \textit{in vitro} and \textit{in vivo}. In the \textit{in vitro} DDI experiment, the uptake of rosuvastatin in PXB-cells was inhibited by cyclosporine A in a concentration-dependent manner, and the observed IC_{50} was 0.21 μmol/L, whereas the reported IC_{50}s of cyclosporine A to OATP1B1/1B3 were 0.2 to 0.9 μmol/L in OATP1B1- and OATP1B3-expressing cells (Hirano et al., 2006; Trieber et al., 2007; Karlgren et al., 2012; Shen et al., 2013). Therefore, we concluded that the observed uptakes of rosuvastatin in PXB-cells were via OATPs, and cyclosporine A inhibited OATPs, as reported previously. In cyclosporine A and other potent inhibitors of OATP1B1 and OATP1B3, pre-incubation enhances its inhibitory effects on these transporters \textit{in vitro} (Shitara et al, 2013 and 2017), and Gartz at al. reported that the inhibitory potency of cyclosporine A against OATP1B1 and OATP1B3 are 0.019 to 0.093 μmol/L with pre-incubation, and 0.198 to 0.411 μmol/L without pre-incubation using transporter-expressing HEK293 cells (Gartz et al., 2013), whereas 0.21 μmol/L was observed value in the study using PXB-mice. When conducting quantitative prediction such as \textit{in vitro-in vivo} extrapolation, it would be more suitable to conduct the \textit{in vitro} DDIs study with pre-incubation via typical transporters like
OATPs. In terms of the species-difference between mouse and human, gene homology of oatps between mice and rats are quite high (Meier-Abt F et al., 2005). In addition, regarding to in vitro DDIs, there are a few reports about inhibition of rodent’s transporters by cyclosporine A in cells, and the IC$_{50}$ of cyclosporine A against rodents’ Oatps, Mrp2 and Mdr1 were similar or slightly higher than those in humans (Kamisako et al., 1999; Shitara et al., 2002; Yasuda et al., 2002). We therefore think that the species-difference in the IC$_{50}$ for these transporters might not cause the different DDI potentials between PXB-mice and SCID mice.

We then investigated in vivo DDIs in PXB-mice. Prior to the interaction study, the pharmacokinetic characteristics of cyclosporine A and rosuvastatin were confirmed in mice. The blood concentration of cyclosporine A reached its maximum rapidly after the oral administration, and the blood concentration-time curve was almost constant from one to eight hours after the oral dose in PXB-mice and SCID mice. The systemic and liver tissue exposures of cyclosporine A were 3- to 5-fold higher in SCID mice than in PXB-mice. Cyclosporine A is mainly metabolized in the liver by CYP3A (Turgeon DK et al., 1992). This may be one of the reasons for the difference in its exposure between SCID mice and PXB-mice; however, we cannot reach a concrete conclusion based on the results obtained in the present study. The systemic exposure of rosuvastatin after its
oral administration was markedly lower in SCID mice than in PXB-mice. Furthermore, we assessed the total systemic clearance of rosuvastatin after its intravenous administration; its total clearance was markedly higher in SCID mice than in PXB-mice, and its blood concentrations just after its intravenous administration approximately 5-fold lower in SCID mice than in PXB-mice. Therefore, the lower systemic exposure of rosuvastatin in SCID mice may be caused by the higher distribution volume of rosuvastatin rather than the higher elimination rate. Iusuf et al. reported that normal mice had low systemic concentrations of rosuvastatin, mainly due to rapid and high liver distribution (Iusuf et al. 2013). In the present study, the liver-to-blood concentration ratio of rosuvastatin was markedly higher in SCID mice than in PXB-mice, which indicates a higher amount of uptake in SCID mice than in PXB-mice. Previous studies also reported that rodent hepatocytes exhibit higher uptake activities for substrates via oatps/OATPs than human hepatocytes (Menochet et al., 2012; Grime and Paine, 2013), and hOATPs-transgenic mice have a weaker uptake potency for statins than control mice (Higgins et al., 2014; Salphati et al., 2014). Based on the characteristics of cyclosporine A and rosuvastatin, we investigated in vivo DDI, and showed that the systemic exposure of rosuvastatin in PXB-mice increased by 3.3- and 11-fold with the pre-administration of cyclosporine A orally at doses of 10 and 50
mg/kg four hours before the administration of 2 mg/kg of rosvastatin. Furthermore, 13- and 78-fold increases in the systemic exposure of rosvastatin were observed in SCID mice with the same conditions in the treatment for cyclosporine A. In a clinical study, a 7.1-fold increase in the systemic exposure of rosvastatin was observed with 75 to 200 mg of cyclosporine treatment in heart transplant patients (Simonson et al, 2004).

The mechanisms for the inhibitory process of an inhibitor against the uptake transporters, OATPs, in the liver have been elucidated in detail. The maximum unbound concentration of an inhibitor at the inlet to the liver ($I_{in, max, u}$) may directly affect its potency to inhibit transporters expressed on the sinusoidal membrane of the liver. Yoshida et al. reported that predicted AUCRs based on $I_{in, max, u}$ were similar to clinically observed AUCRs within a two- to three-fold error margin in 44/52 studies (Yoshida et al., 2012). In the present study, we calculated $I_{in, max, u}$ in each dosing case in PXB-mice and SCID mice in order to evaluate comparability between calculated $I_{in, max, u}$ and observed AUCRs. With the pre-administration of cyclosporine A at a dose of 10 mg/kg, 3.3- and 13-fold increases in the systemic exposure of rosvastatin were observed, with $I_{in, max, u}$ of 0.29 to 0.94 and 0.36 to 0.96 μmol/L of cyclosporine A in PXB-mice and SCID mice, respectively. When the dosage of cyclosporine A increased to 50 mg/kg, AUCRs were 11 and 78 with $I_{in, max, u}$ of 1.4 to 4.7 and 1.5 to 4.8 μmol/L of cyclosporine.
A in PXB-mice and SCID mice, respectively. Although the observed AUCRs markedly differed between PXB-mice and SCID mice at the same dose level of cyclosporine A, both $I_{\text{in, max, u}}$ were close to each other. Clinical findings reported showed that when a 7.1-fold increase was observed, estimated $I_{\text{in, max, u}}$ was 1.2 to 1.3 $\mu\text{mol/L}$ (Yoshida et al, 2012; Izumi et al. 2015). Therefore, the observed AUCRs of rosuvastatin in PXB-mice in the present study may be reasonable and in accordance with the estimated $I_{\text{in, max, u}}$ of cyclosporine A. On the other hand, the 78-fold increase in the systemic exposure of rosuvastatin in SCID mice with 50 mg/kg of cyclosporine A requires scientific and biological explanations, and we found and focused on the high concentrations of cyclosporine A in the liver. Eight hours after the oral administration of 50 mg/kg of cyclosporine A (at the same time as four hours after the oral administration of rosuvastatin), liver concentrations were 30.0 $\mu\text{mol/kg tissue}$ in SCID mice and 5.89 $\mu\text{mol/kg tissue}$ in PXB-mice. Rosuvastatin is known to be excreted into bile via transporters expressed on the apical membrane of hepatocytes, e.g. MRP2 and BCRP (Huang et al., 2006; Ellis et al., 2013), and the reported IC$_{50}$ of cyclosporine A against MRP2 and BCRP were 9.3 and 1.5 $\mu\text{mol/L}$, respectively (Yoshida et al, 2012). The mechanisms by which an inhibitor acts toward transporters expressed on the apical membrane inside hepatocytes have not been elucidated; however, the results of the
present study indicate that high concentrations of cyclosporine A in the liver affect the pharmacokinetics of rosuvastatin and increase its systemic exposure by 78-fold in SCID mice (Fig. 4). In addition, we have additionally investigated concentrations of hydroxy form of cyclosporine A, a potential inhibitor of OATPs, the concentrations of the metabolite in both blood and liver were very high, which was at the same level as unchanged form (Supplemental Table 3). Since the inhibitory potency in vitro of the metabolite, cyclosporine AM1 against OATPs has been reported at the same level as unchanged form (Gartz et al., 2013), its inhibitory potency in vivo of cyclosporine AM1 should be noticed to estimate quantitative relationship between concentrations of those inhibitors and AUCRs of victim rosuvastatin observed. To assess intestinal DDIs, we additionally conducted in vivo DDI experiment by the intravenous administration of rosuvastatin with the pretreatment of 10 and 50 mg/kg cyclosporine A in PXB-mice, and observed AUCRs were 2.5 and 6.2, respectively (Supplemental Table 1), whereas 3.3 and 11 by the oral administration of rosuvastatin. Therefore, the AUCRs via the intestinal DDIs would be considered to be 1.3 at the dose of 10 mg/kg cyclosporine A and 1.8 at the dose of 50 mg/kg cyclosporine A. We think that the magnitude of hepatic portion to total DDIs are still major, but there was some level of intestinal DDIs occurred between rosuvastain and cyclosporine A, especially at the higher cyclosporine
A-dose. Takahashi et al. reported the possibility of the intestinal DDIs between pitavastatin and cyclosporine A in cynomolgus monkeys (Takahashi et al., 2013). As described above, cyclosporine A is known to inhibit not only OATPs but BCRP and MRP2, which are also transporters rosuvastain is taken by (Fig. 4). The intestinal DDIs might be one of limiting factors for usage of the chimeric mice with humanized liver to estimate human DDIs accurately because of the lack of the human gastrointestinal condition. The explorations of dose-dependency of an inhibitor and its magnitude to gastrointestinal transporters should be conducted when using the chimeric mice for hepatic DDIs studies.

In the present study, we also measured the metabolites of rosuvastatin in order to identify the replacement of human livers in PXB-mice, and assess the inhibitory effects of cyclosporine A on the metabolism of rosuvastatin. Rosuvastatin is known to be absorbed (~50%) and largely excreted in its unchanged form into bile (Martin et al, 2003a, 2003b). In addition, the lactone form (UGT1A metabolite) and N-desmethyl form (CYP2C metabolite) have also been reported as the metabolites of rosuvastatin in clinical studies (Prueksaritanont et al., 2002; Martin et al, 2003b). In the present study, these two metabolites were monitored, and the lactone form was detected in the livers of PXB-mice, while the N-desmethyl form was also detected in the plasma and livers of
PXB-mice. However, these metabolites were hardly detected in SCID mice. These results suggest that these metabolites may be human-specific or abundant metabolites. In the \textit{in vivo} DDIs experiments of the present study, because the exposure of these two metabolites in PXB-mice was markedly lower than the increased AUCs of its unchanged form of rosvastatin, and there were almost no effects on the concentrations of these metabolites with or without the administration of cyclosporine A, we estimate that \textit{in vivo} DDI observed in the present study was considered to be mainly due to a transporter-mediated phenomenon, not a metabolism-mediated one. In conclusion, the results in this study indicate that PXB-mice might be a useful tool for predicting human OATPs-mediated DDIs, and also its limitation due to the differences of gastrointestinal condition from human should be considered. We recommend that the mice would be quite useful when we are estimating a potency of a drug candidate as an inhibitor on any drug-drug interaction via human hepatic transporters; However, a probe compound (victim) should be administered not only orally but intravenously to estimate its inhibitory magnitude of a candidate to gastrointestinal transports. In addition, it would be better for us to conduct those studies in different dose levels of a candidate to know its linearity/proportionality of the \textit{in vivo} DDIs.
Acknowledgements

The authors thank Tatsuya Matsumi, Tomoko Watadani, and other members in PhoenixBio Co. Ltd. for providing PXB-cells, PXB-mice, and other materials derived from PXB-mice.

Authorship Contributions

Participation in research design: Uchida and Tajima.
Conducted experiments: Tajima, Uchida, Kakuni, Kageyama, Okada, and Sakurada.
Performed data analysis: Uchida and Tajima.
Wrote or contributed to the writing of the manuscript: Uchida, Tajima, Kakuni, Tateno and Hayashi.
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*Drug Metab Dispos* **42**:1055-1065.


*Drug Metab Dispos* **42**:1067-1073.


Committee for Human Medicinal Products (CHMP) (2012) Guideline on the Investigation of Drug Interactions [Final], European Medicines Agency


Pharmaceuticals and Medical Devices Agency (PMDA) (2014) Drug interaction guideline for drug development and labeling recommendations [Final Draft]


Footnotes

Masashi Uchida and Yoriko Tajima contributed equally to this work.
Figure Legends

Fig. 1 *In vitro* uptake studies of rosuvastatin; (a) Time-dependent uptake of rosuvastatin in PXB-cells. Rosuvastatin was incubated with cells at the concentration of 1 μmol/L both at 37°C and on ice up to ten minutes.; (b) Concentration-dependent uptake of rosuvastatin in PXB-cells. Rosuvastatin was incubated with cells at the concentration from 0.1 to 30 μmol/L both at 37°C and on ice for ten minutes. By subtracting each value obtained on ice from corresponding value at 37°C, an active uptake amount of rosuvastatin was calculated at each time point.; (c) Concentration-dependent inhibition of cyclosporine A against the uptake of rosuvastatin in PXB-cells. Rosuvastatin was incubated with cells at the concentration of 1 μmol/L at 37°C for ten minutes with or without the inhibitor, cyclosporine A. The concentrations of the inhibitor were from 0.01 to 3 μmol/L. Values shown are the mean ± S.D. for experiments performed in triplicate.

Fig. 2 Blood concentration-time profiles of rosuvastatin after the oral administration of rosuvastatin at a dose of 2 mg/kg to male PXB-mice (a) and SCID mice (b), with pre-administration orally at doses of 10, and 50 mg/kg of cyclosporine A or vehicle. Open circles represent the group of vehicle (control); solid circles represent the group of 50 mg/kg of cyclosporine A; solid triangles represent the group of 10 mg/kg of
cyclosporine A. Each point is represented as mean ± S.D. (n=3 to 4).

Fig. 3 Liver-to-blood concentration ratios of rosuvastatin at four hours after oral administration of 2 mg/kg of rosuvastatin to male PXB-mice (a) or SCID mice (b) with oral pre-administration of the doses of 10, 50 mg/kg of cyclosporine A. Each point is represented as mean ± S.D. (n=3 to 4). *P < 0.05, statistically significant compared with control (vehicle) group.

Fig. 4 Estimated mechanism of *in vivo* drug-drug interaction between rosuvastatin and cyclosporine A via transporters (absorbed rosuvastatin is uptaken by multiple transporters expressed in the sinusoidal membrane of human hepatocytes, e.g. OATPs, and excreted via other transporters expressed on the apical membrane inside hepatocytes, e.g. BCRP, and MRP2) (a). The uptake and efflux of rosuvastatin were inhibited by the cyclosporine A coadministered, and subsequently the systemic exposure of rosuvastatin would increase (b).
Tables

Table 1 Pharmacokinetic parameters of rosuvastatin after oral (2 mg/kg) and intravenous (1 mg/kg) administrations of rosuvastatin to male PXB-mice and SCID mice, and effect of oral pre-administration of cyclosporine A on systemic exposure of rosuvastatin in the both mice

<table>
<thead>
<tr>
<th>Cyclosporine A (mg/kg)</th>
<th>PXB-mice</th>
<th>SCID mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>506 ± 126*</td>
<td>196 ± 26*</td>
</tr>
<tr>
<td>AUC$_{0-4\text{h, oral}}$ (ng h/mL)</td>
<td>1162 ± 315*</td>
<td>344 ± 84*</td>
</tr>
<tr>
<td>$C_{\text{0, iv}}$ (ng/mL)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AUC$_{0-4\text{h, iv}}$ (ng h/mL)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$CL_{\text{ext}}$ (L/h/kg)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$V_d$ (L/kg)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$V_{d,ss}$ (L/kg)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AUCR</td>
<td>11</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. from three or four animals. AUCR = AUC$_{0-4\text{h, oral}}$ with pretreatment of cyclosporine A / AUC$_{0-4\text{h, oral}}$ with pretreatment of vehicle (control). $V_d = \text{Dose} / C_{\text{0, iv}}$. $*P < 0.05$, statistically significant compared with control (vehicle) group.
Table 2: Estimation of maximum protein-unbound concentration of cyclosporine A at the inlet to the liver, and observed AUCRs in drug-drug interaction studies

<table>
<thead>
<tr>
<th></th>
<th>Rosuvastatin</th>
<th>Dose (mg/kg)</th>
<th>F_dF_g</th>
<th>k_a (min⁻¹)</th>
<th>Q_h (mL/min)</th>
<th>f_u,b (%)</th>
<th>I_{in,max,u} (μmol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PXB-mice</td>
<td>3.3</td>
<td>10</td>
<td>0.3 - 1.0</td>
<td>0.1</td>
<td>1.8</td>
<td>10</td>
<td>0.29 - 0.94</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>50</td>
<td>0.3 - 1.0</td>
<td>0.1</td>
<td>1.8</td>
<td>10</td>
<td>1.4 - 4.7</td>
<td></td>
</tr>
<tr>
<td>SCID mice</td>
<td>13</td>
<td>10</td>
<td>0.3 - 1.0</td>
<td>0.1</td>
<td>1.8</td>
<td>10</td>
<td>0.36 - 0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>50</td>
<td>0.3 - 1.0</td>
<td>0.1</td>
<td>1.8</td>
<td>10</td>
<td>1.5 - 4.8</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>7.1</td>
<td>200⁺</td>
<td>1.0</td>
<td>0.1</td>
<td>1450</td>
<td>10</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1620</td>
<td>11</td>
<td>1.2</td>
<td>Izumi et al., 2015</td>
</tr>
</tbody>
</table>

* a mg/body
Table 3 Blood and tissue concentrations in the liver of cyclosporine A after oral administration of doses of 10 and 50 mg/kg of cyclosporine A in PXB-mice and SCID mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Dose (mg/kg)</th>
<th>Systemic exposure</th>
<th>Liver tissue exposure</th>
<th>Inlet to liver</th>
<th>Reported IC_{50} (μmol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C_{max} (μmol/L)</td>
<td>AUC (μmol·h/L)</td>
<td>C_{in,max} (μmol/kg tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PXB-mice</td>
<td>10</td>
<td>0.131</td>
<td>0.292</td>
<td>1.07</td>
<td>0.29 - 0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.349</td>
<td>1.02</td>
<td>5.89</td>
<td>1.4 - 4.7</td>
<td></td>
</tr>
<tr>
<td>SCID mice</td>
<td>10</td>
<td>0.402</td>
<td>1.20</td>
<td>4.95</td>
<td>0.36 - 0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1.58</td>
<td>5.25</td>
<td>30.0</td>
<td>1.5 - 4.8</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean from three or four animals.

a Corresponding to liver concentrations 4 hours after oral administration of rosvustatin.

Yoshida et al., 2012
Fig. 1

Panel a: Time course of Rosuvastatin production at 37°C and on ice.

Panel b: Concentration of Rosuvastatin at different concentrations.

Panel c: Effect of CsA concentration on Rosuvastatin production.
Supplemental Data

Journal Title

Drug Metabolism and Disposition

Article Title

Organic anion transporting polypeptides (OATPs)-mediated drug-drug interaction study between rosvastatin and cyclosporine A in chimeric mice with humanized liver.

Authors

Masashi Uchida, Yoriko Tajima, Masakazu Kakuni, Yutaka Kageyama, Taro Okada, Eri Sakurada, Chise Tateno, Ryoji Hayashi
**Supplemental Table 1.** Pharmacokinetic parameters of rosuvastatin after intravenous (1 mg/kg) administrations of rosuvastatin to male PXB-mice and SCID mice, and effect of oral pre-administration of cyclosporine A on systemic exposure of rosuvastatin in the both mice

<table>
<thead>
<tr>
<th>Cyclosporine A (mg/kg)</th>
<th>PXB-mice</th>
<th>SCID mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>$C_{0,iv}$ (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3235 ± 952*</td>
<td>1971 ± 629*</td>
</tr>
<tr>
<td>$AUC_{0,4h,iv}$ (ng·h/mL)</td>
<td>1743 ± 518*</td>
<td>711 ± 172*</td>
</tr>
<tr>
<td>$CL_{tot}$ (L/h/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.541 ± 0.162*</td>
<td>1.33 ± 0.27*</td>
</tr>
<tr>
<td>$V_d$ (L/kg)</td>
<td>0.329 ± 0.091*</td>
<td>0.549 ± 0.178*</td>
</tr>
<tr>
<td>$V_{d,ss}$ (L/kg)</td>
<td>0.859 ± 0.426*</td>
<td>1.55 ± 0.73*</td>
</tr>
<tr>
<td>$AUCR_{iv}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. from three or four animals.

$AUCR_{iv} = AUC_{0,4h,iv}$ with pretreatment of cyclosporine A / $AUC_{0,4h,iv}$ with pretreatment of vehicle (control)

$V_d = \text{Dose} / C_{0,iv}$

* $P < 0.05$, statistically significant compared with control (vehicle) group

**Supplemental Table 2.** Fraction unbound of cyclosporine A in blood of PXB-mice

<table>
<thead>
<tr>
<th>CsA Concentration (μmol/L)</th>
<th>$f_{up}$ (%)</th>
<th>$f_p$ (%)</th>
<th>RB</th>
<th>$f_{ub}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>10.4</td>
<td>89.7</td>
<td>2.14</td>
<td>4.86</td>
</tr>
<tr>
<td>2.0</td>
<td>16.8</td>
<td>83.3</td>
<td>1.64</td>
<td>10.3</td>
</tr>
</tbody>
</table>

$\text{f}_{up}$: fraction unbound in plasma

$f_p$: fraction bound in plasma

$RB$: blood to plasma concentration ratio

$\text{f}_{ub}$: fraction unbound in blood
**Supplemental Table 3.** Blood and tissue concentrations of hydroxy form of cyclosporine A, cyclosporine AM1, in the liver after oral administration of doses of 10 and 50 mg/kg of cyclosporine A in PXB-mice and SCID mice.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Systemic exposure</th>
<th>Liver tissue exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( C_{\text{max}} ) (( \mu \text{mol/L} ))</td>
<td>AUC (( \mu \text{mol} \cdot \text{h/L} ))</td>
</tr>
<tr>
<td>PXB-mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.150</td>
<td>0.314</td>
</tr>
<tr>
<td>50</td>
<td>0.340</td>
<td>0.830</td>
</tr>
<tr>
<td>SCID mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.386</td>
<td>0.771</td>
</tr>
<tr>
<td>50</td>
<td>1.08</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Data are presented as mean from three or four animals.

\( a \) Corresponding to liver concentrations 4 hours after oral administration of rosuvastatin.