In Vitro–In Vivo Extrapolation of OATP1B-Mediated Drug–Drug Interactions in Cynomolgus Monkey


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ABSTRACT

Hepatic organic anion-transporting polypeptides (OATP) 1B1 and 1B3 are clinically relevant transporters associated with significant drug–drug interactions (DDIs) and safety concerns. Given that OATP1B1s in cynomolgus monkey share >90% degree of gene and amino acid sequence homology with human orthologs, we evaluated the in vitro–in vivo translation of OATP1B-mediated DDI risk using this preclinical model. In vitro studies using plated cynomolgus monkey hepatocytes showed active uptake Km values of 2.0 and 3.9 μM for OATP1B probe substrates, pitavatatin and rosuvastatin, respectively. Rifampicin inhibited pitavatatin and rosuvastatin active uptake in monkey hepatocytes with IC50 values of 3.0 and 0.54 μM, respectively, following preincubation with the inhibitor. Intravenous pharmacokinetics of 3H2-pitavatatin and 3H2-rosuvastatin (0.2 mg/kg) and the oral pharmacokinetics of cold probes (2 mg/kg) were studied in cynomolgus monkeys (n = 4) without or with coadministration of single oral ascending doses of rifampicin (1, 3, 10, and 30 mg/kg). A rifampicin dose-dependent reduction in i.v. clearance of statins was observed. Additionally, oral pitavatatin and rosuvastatin plasma exposure increased up to 19- and 15-fold at the highest dose of rifampicin, respectively. Use of in vitro IC50 obtained following 1 hour preincubation with rifampicin (0.54 μM) predicted correctly the change in mean i.v. clearance and oral exposure of statins as a function of mean unbound maximum plasma concentration of rifampicin. This study demonstrates quantitative translation of in vitro OATP1B IC50 to predict DDIs using cynomolgus monkey as a preclinical model and provides further confidence in application of in vitro hepatocyte data for the prediction of clinical OATP1B-mediated DDIs.

Introduction

Drug–drug interactions (DDIs) involving hepatic organic anion-transporting polypeptides (OATPs) are widely recognized as clinically important due to potential serious adverse events associated with them (Giacomini et al., 2010; Yoshida et al., 2012; El-Kattan et al., 2016; Galetin et al., 2017). Therefore, there is a strong need to assess OATP-mediated DDI risk early in candidate identification and drug development. Despite tremendous strides in establishing in vitro tools for assessing transporter role, confidence in quantitative prediction of transporter-mediated DDIs using in vitro data is arguably still low to moderate (Zamek-Gliszczynski et al., 2013; Jones et al., 2015; Yoshida et al., 2017). Recently, cynomolgus monkey has been increasingly evaluated as a potential animal model for the assessment of OATP1B1-mediated DDIs (Shen et al., 2013, 2015; Takahashi et al., 2013, 2016; Chu et al., 2015) due to >90% degree of gene and amino acid sequence homology between cynomolgus monkey and human orthologs for OATP1B uptake transporters (Ebeling et al., 2011; Shen et al., 2013; Takahashi et al., 2013). In addition to clinical drug probes, increasing evaluation of endogenous biomarkers for OATP1B DDIs (e.g., coproporhyrin I, bile acids) has been reported in this preclinical species (Chu et al., 2015; Watanabe et al., 2015; Shen et al., 2016; Thakare et al., 2017). DDIs reported in cynomolgus monkey for statins, including rosuvastatin, pitavastatin, and atorvastatin, showed a good agreement in DDI classification (strong/moderate) relative to those observed in humans
Ki) to in vivo was reported for OATP inhibitors (Varma et al., 2015). Nevertheless, an in vitro–in vivo extrapolation (IVIVE) strategy using either static or physiologically-based pharmacokinetic modeling approaches accounting for the species differences could be employed for predicting clinical DDI risk.

Discrepancy in the translation of inhibitory potency (IC50 or Ki) to in vivo was reported for OATP inhibitors (Varma et al., 2012; Li et al., 2014; Yoshikado et al., 2017). For example, rifampicin in vitro Ki values were shown to be several fold higher than the in vivo Ki estimated from the clinical DDI data using mechanistic modeling (Varma et al., 2014; Barnett et al., 2017; Yoshikado et al., 2017). In addition, a number of studies demonstrated potentiation of OATP1B inhibition following preincubation with the inhibitor, trend particularly evident for cyclosporine (Amundsen et al., 2010; Gertz et al., 2013; Izumi et al., 2015; Takahashi et al., 2016; Pawha et al., 2017) and incorporated in the recent Food and Drug Administration DDI guidance document (https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf). Although the clinical DDIs with cyclosporine are well recovered with the IC50 obtained following preincubation (Gertz et al., 2013), there is limited understanding of the significance of preincubation in predicting DDIs of other inhibitor drugs. Additionally, recent studies reported substrate dependence in the in vitro inhibition data for OATP1B inhibitors (Noé et al., 2007; Izumi et al., 2013). Due to the gaps in the available clinical DDI data, understanding of the translation of preincubation and substrate-dependent inhibition in vitro phenomena is still ambiguous.

Using cynomolgus monkey as a preclinical model, our goals in the current study are as follows: 1) to evaluate the predictability of OATP1B-mediated DDIs from in vitro inhibition data and 2) to understand the in vitro relevance of the effect of preincubation and substrate dependency in rifampicin inhibition potential measured in vitro. In this study, the in vitro inhibition of transporter-mediated uptake of rosuvastatin and pitavastatin was investigated after incubation of plated cynomolgus monkey primary hepatocytes either with buffer or OATP inhibitors (rifampicin, cyclosporine, and rifamycin SV). Prior to inhibition studies, an uptake kinetic characterization was performed for these two statins. Pitavastatin and rosuvastatin represent extended clearance classification system class 1B and class 3B, respectively, in which OATP-mediated hepatic uptake is the rate-determining step in the systemic clearance in human (Varma et al., 2015, 2017). To allow separate evaluation of inhibitory effect of hepatic versus intestinal disposition, pharmacokinetics of both statins were studied following simultaneous i.v. (stable-labeled) and oral (cold) administration to cynomolgus monkey (n = 4 animals) and over a wide rifampicin dose range (1–30 mg/kg). Finally, the inhibition data obtained using monkey hepatocytes to the in vivo changes in systemic clearance and plasma (i.v/oral) exposure of statins was evaluated.

Materials and Methods

Chemicals and Reagents. The 3′-phosphoadenosine-5′-phosphosulfate, simvastatin, rifamycin SV, rifampicin, 1-aminobenzotriazole, cyclosporine A, naloxone, and tolbutamide were purchased from Sigma-Aldrich (St. Louis, MO). Deuterium-labeled rifampicin (2H8-rifampicin) was obtained from ALSACHIM (Illkirch, Graffenstaden, France). Pitavastatin and rosuvastatin were purchased from Sequoia Research Products (Oxford, UK). Deuterium-labeled pitavastatin (2H9-pitavastatin) and rosuvastatin (2H6-resuvastatin) were purchased from Clearsynth (Ontario, Canada).

Atorvastatin was purchased from Toronto Research Chemicals (Toronto, Canada). InVitroGro CP hepatocyte medium and Torpedo antibiotic mix were purchased from InVitro GmbH (Frankfurt, Germany). Collagen I–coated 24-well plates were obtained from VWR International (Leicestershire, UK). Cryopreserved cynomolgus monkey hepatocytes (female, pooled lot 10353012) were purchased from In Vitro ADMET Laboratories (Columbia, MD). Bicinchoninic acid protein assay kit was purchased from Life Technologies (Paisley, UK). Dulbecco’s phosphate-buffered saline (DPBS) was obtained from Life Technologies, Acetonitrile, water, and ammonium hydroxide were obtained from Fisher Scientific (Fair Lawn, NJ). Methanol was purchased from Fisher Scientific and VWR International.

In Vitro Transport Studies Using Monkey Hepatocytes. Cryopreserved cynomolgus monkey hepatocytes were thawed in prewarmed InVitroGro CP medium supplemented with torpedo antibiotic mix (2.2% v/v), according to the protocol from the protocol from InVitro GmbH, and cell viability was determined by trypan blue exclusion method. Hepatocyte suspension was diluted to 0.7 × 106 cells/ml with the prepared InVitroGro CP medium, and hepatocytes were seeded into collagen I–coated 24-well plates at a density of 350,000 cells/well. Cells were cultured for 4 hours at 37°C and 5% CO2 in an incubator to allow attachment to the collagen. Cell confluency and monolayer formation were visually assessed before each experiment. Both the hepatic transporter uptake and its inhibition were investigated in plated cynomolgus monkey hepatocytes 4 hours postseeding using rosuvastatin and pitavastatin as probe substrates. Uptake was measured over a range of concentrations (0.1–30 μM) for 30, 60, 90, and 120 seconds at 37°C in triplicate to determine uptake kinetics, as described previously (Ménochet et al., 2012). Inhibition of rosuvastatin and pitavastatin hepatic uptake by monkey hepatocytes was assessed in triplicate using the OATP inhibitor rifampicin (0.01–100 μM). In addition, inhibition with cyclosporine (0.01–6 μM) and rifamycin SV (0.01–100 μM) was investigated. Cyclosporine was included as a strong OATP inhibitor with evidence of preincubation effect in human in vitro systems (Gertz et al., 2013; Izumi et al., 2015), whereas rifamycin SV was considered as dual inhibitor of OATPs and NTCP (Bi et al., 2017). Rosuvastatin and pitavastatin concentrations used in the inhibition studies were 1 and 0.3 mM, respectively. The medium was removed after plating, and cell monolayers were rinsed twice with prewarmed DPBS. Effect of preincubation on the inhibition of hepatic uptake transporters in cynomolgus monkey hepatocytes was investigated by the addition of an inhibitor solution (400 μM) on the cell monolayer for 1 hour.

In control group, preincubation was performed with DPBS containing 1 mM 1-aminobenzotriazole. Following preincubation, cell monolayers were coincubated with prewarmed DPBS containing the inhibitor and the probe substrate for 3 minutes. This incubation was stopped by the removal of the medium and rinsing of the cell monolayers three times with 800 μl ice-cold DPBS. The cell monolayers were lysed in 200 μl ice-cold deionized water for subsequent liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. The in vitro sample preparation and LC-MS/MS analysis...
are described in Supplemental Material, Section 1. The LC-MS/MS conditions for each individual drug and their corresponding internal standards are detailed in Supplemental Table 2.

**In Vitro Data Analysis.** Determination of the uptake kinetic parameters, including the affinity constant $K_a$ (μM), the maximum uptake rate $V_{\text{max}}$ (pmol/min/10^6 cells), passive diffusion clearance ($C_{\text{diff}}$, microliters per minute per 10^6 cells), and fraction unbound in the cell fraction of unbound drug in the cell for rosuvastatin and pitavastatin, was performed using the two-compartment mechanistic model in Matlab (2015a) (MathWorks, Natick, MA), as described previously (Ménocet et al., 2012). The active uptake clearance ($C_{\text{transp}}$, μl/min/10^6 cells) was estimated from the $V_{\text{max}}$ to $K_a$ ratio. Parameter estimates were corrected for nonspecific binding in the media ($f_{\text{media}}$), which was calculated from the slope of the linear regression of the unbound substrate concentration extrapolated at $t = 0$ versus the initial media concentration curve. The cellular concentrations were normalized for protein content as measured by the bicinchoninic acid protein assay kit at the end of incubation. Hepatocyte volume was set to 3.9 μl/10^6 cells as in rat (Reinoso et al., 2001); conversion of monkey hepatocyte data expressed per mg protein to M cells was based on the 1:1 relationship between milligram protein and M cells (in-house data).

The data on substrate uptake (expressed as percentage of control) at each inhibitor concentration were used to estimate the $IC_{50}$ (μM) of the inhibitors used. The analysis was performed in GraFit v6.0 (Erithacus Software, Horley, UK) by fitting a nonlinear least squares regression model, as shown in eq. 1 to the experimental data:

$$\text{Substrate uptake} (\% \text{ control}) = \frac{\text{Max} - \text{Min}}{1 + \left(\frac{IC_{50}}{I}\right)^s} + \text{Min} \tag{1}$$

where Max and Min represent the fitted maximum and minimum uninhibited uptake, respectively, $I$ is the inhibitor concentration, corrected for $f_{\text{media}}$ (0.85, 0.7, and 0.95 for rifampicin, cyclosporine, and rifamycin SV, respectively), and $s$ is the slope factor. To increase the precision of the $IC_{50}$ estimates, the minimum uptake was fixed to the experimental data. In all cases, the experimental data for minimum uptake were within 25% of the estimated value. Statistical analysis of the preincubation effect on $IC_{50}$ was performed using a paired t test, where $p < 0.05$ was considered as statistically significant.

**In Vivo Studies in Cynomolgus Monkeys.** All procedures performed on these animals were in accordance with regulations and established guidelines, were reviewed and approved by Pfizer Institutional Animal Care and Use Committee, and were conducted at Pfizer (Groton, CT). Male cynomolgus macaque Mauritian monkeys (approximately 6–8 years of age) were used for these studies. A crossover study design was employed, in which the same four animals were dosed over a series of five studies, following a minimum 1-week washout period between each study. One exception was the 3 mg/kg rifampicin dose group, in which one of four monkeys was dosed only in that single study. Animals were provided a normal food schedule the day before the study (meals at 8:00 AM and 11:00 AM, with one treat daily) and were allowed free access to water. Animals were housed in metabolism cages during sample collection. On the day of the study, monkeys were fed at approximately 1 and 3 hours postdose and allowed water ad libitum. Rifampicin was administered via oral gavage at 0 (blank vehicle), 1, 3, 10, and 30 mg/kg, at a dose volume of 2 ml/kg in a 0.5% (w/v) methylcellulose (in water) suspension. Rifampicin administration was immediately followed by oral doses of pitavastatin and rosuvastatin at a dose of 2 mg/kg. Approximately 1 hour and 15 minutes following the oral rifampicin administration, $^{3}H_{\text{H}}$-pitavastatin and $^{3}H_{\text{H}}$-rosuvastatin were administered via i.v. bolus (cephalic vein), at a dose of 0.2 mg/kg, in a dosing volume of 0.2 ml/kg; 2% dimethyl sulfoxide (v/v); and 98% of Tris-buffered saline (pH 7.7). All i.v. formulations were sterile filtered prior to administration. Serial blood samples were collected via the femoral vein into K$_2$EDTA tubes prior to dosing and then at 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 5, 6, and 24 hours post i.v. dosing. Blood samples were stored on wet ice prior to being centrifuged to obtain plasma (3000 rpm, 10 minutes at 4°C; Jouan BR4i refrigerated centrifuge). Urine was also collected on wet ice predose and at intervals of 0–6 and 6–24 hours postdose. Due to the potential instability of rifampicin and possible interconversion of lactone and acid forms of pitavastatin or rosuvastatin, each plasma and urine sample was equally divided into two aliquots prior to being stored frozen. The first aliquot was untreated matrix, whereas the second aliquot was added to an equal volume of 0.1 M sodium acetate buffer (pH 4). All urine and plasma samples, treated and untreated, were kept cold during collection, after which they were stored frozen at –20°C. The analysis of $^{3}H_{\text{H}}$-pitavastatin, $^{3}H_{\text{H}}$-rosuvastatin, pitavastatin, rosuvastatin, and rifampicin in plasma samples by LC-MS/MS was described in Supplemental Material, Section 2. The analytes were monitored using multiple reaction monitoring with settings listed in Supplemental Table 3.

**Pharmacokinetic Analysis and DI Prediction.** Analyst 1.4.2 software (SCIEX, Framingham, MA) was used for LC-MS/MS peak integration of plasma and urine samples. Raw data were imported into Watson LIMS version 7.4 (Thermo Fisher Scientific, Waltham, MA) for standard curve regression and noncompartmental pharmacokinetic parameter calculations—area under the plasma concentration–time curve (AUC), maximum plasma concentration ($C_{\text{max}}$), i.e. clearance ($C_{\text{L}}$), volume of distribution ($V_{\text{dss}}$), and half-life ($t_{1/2}$). Other parameters were subsequently calculated on the basis of pharmacokinetic first principles. Oral bioavailability ($F$) of statins was estimated by eq. 2:

$$F = F_a F_g F_h$$

where $F_a$ represents fraction of drug absorbed; $F_g$, fraction of drug escaping intestinal extraction; and $F_h$, fraction of drug escaping hepatic extraction. The $F_h$ was estimated by eq. 3:

$$F_h = 1 - \frac{C_{\text{Lhepatic}}}{Q_h}$$

where $C_{\text{Lhepatic}}$ represents hepatic blood clearance (plasma $C_{\text{Lhepatic}}$/blood-to-plasma ratio) and $Q_h$ is hepatic blood flow in cynomolgus monkey (44 ml/min/kg) (Hoese et al., 2009). Measured blood-to-plasma ratios of rosuvastatin and pitavastatin in cynomolgus monkey were 0.55 and 0.58, respectively.

The ratio of the AUC (AUCR) of oral statins in the presence (AUC$_{\text{po}}$) and absence (AUC$_{\text{po}}$) of rifampicin was predicted based on eq. 4:

$$\text{AUCR} = \frac{\text{AUCR}_{\text{po}}}{\text{AUCR}_{\text{po}}} = \frac{F_h' F_g' F_a'}{F_h F_g F_a} \times \frac{C_{\text{Lhepatic}}'}{C_{\text{Lhepatic}}} \tag{4}$$

where $'$ indicates parameters in the presence of rifampicin. For the prediction purposes, it was assumed that rifampicin has no impact on $F_a$ and $F_h$ of both statins, and therefore $F_a'$ and $F_h'$ were the same as in the control phase. $F_h'$ was estimated from the hepatic blood clearance in the presence of rifampicin ($C_{\text{Lhepatic}}'$), as shown in eq. 5:

$$F_h' = 1 - \frac{C_{\text{Lhepatic}}'}{Q_h} \tag{5}$$

The $C_{\text{Lhepatic}}$ of statins is the sum of hepatic and renal clearance, and, assuming uptake is the rate-determining step for hepatic clearance, it can be expressed as shown in eq. 6:

$$C_{\text{Lhepatic}} = C_{\text{Lhepatic}} + C_{\text{Lrenal}} = C_{\text{Lactive}} + C_{\text{Ldiff}} + C_{\text{Lrenal}} \tag{6}$$

where $C_{\text{Lrenal}}$, $C_{\text{Lactive}}$, and $C_{\text{Ldiff}}$ represent renal, sinusoidal active uptake, and passive diffusion clearance, respectively. In vivo $IC_{50}$ values were estimated using eq. 7 using unbound $C_{\text{max}}$ of rifampicin ($C_{\text{u,max}}$) as independent variable and i.v. clearance of statins as the depending variable.
\[
\text{CL}_{\text{IV}}' = \left[ \frac{\text{CL}_{\text{active}}}{1 + \frac{\text{IC}_{50}}{\text{CL}_{\text{renal}}}} + \text{CL}_{\text{diff}}' \right] + \text{CL}_{\text{renal}}'
\]

$\text{CL}_{\text{diff}}'$ was obtained from the data in the presence of the highest rifampicin dose, assuming complete inhibition of active hepatic uptake (eq. 8). It was assumed that rifampicin had no impact on the passive diffusion and renal clearance of both statins, and therefore $\text{CL}_{\text{diff}}'$ and $\text{CL}_{\text{renal}}'$ were the same as $\text{CL}_{\text{diff}}$ and $\text{CL}_{\text{renal}}$.

\[
\text{CL}_{\text{diff}} = \text{CL}_{\text{iv}} + \frac{\text{IC}_{50}}{\text{CL}_{\text{renal}}} \cdot \text{CL}_{\text{renal}}
\]

Geometric mean fold error (gmfe) was calculated to assess the bias of the predicted rosuvastatin and pitavastatin AUC across rifampicin dose range, as shown in eq. 9 (Gertz et al., 2010):

\[
\text{gmfe} = 10^{\frac{1}{N} \sum_{j=1}^{N} \log_{10} \left( \frac{\text{predicted}}{\text{observed}} \right)}
\]

where $N$ represents the number of observations.

**Results**

**In Vitro Uptake Kinetics of Rosuvastatin and Pitavastatin in Monkey Hepatocytes.** Uptake of rosuvastatin and pitavastatin (0.1–100 µM) was investigated after incubation with plated cynomolgus monkey hepatocytes, and the kinetic parameters were estimated using the mechanistic two-compartment model. The measured $f_{u,\text{med}}$ was 0.83 and 0.99 for rosuvastatin and pitavastatin, respectively, which was used for correction of the initial media concentrations of both drugs. Kinetic profiles of both drugs in monkey hepatocytes demonstrated a saturable and nonsaturable uptake phase (Fig. 1; Table 1). The $K_{m}$ of rosuvastatin and pitavastatin were 3.29 and 1.99 µM, respectively. Although pitavastatin active uptake clearance was greater than that for rosuvastatin ($\text{CL}_{\text{active}}$ of 109 µl/min/10⁶ cells versus 98.3 µl/min/10⁶ cells), the contribution of active process to total uptake was approximately 96% in case of rosuvastatin versus 80% for pitavastatin. The $\text{CL}_{\text{diff}}$ was approximately 6-fold higher for pitavastatin (26.5 µl/min/10⁶ cells) than in the case of rosuvastatin (4.27 µl/min/10⁶ cells). Furthermore, the extent of intracellular binding differed between the two probes, as fraction of unbound drug in the cell was 0.25 and 0.024 for rosuvastatin and pitavastatin, respectively.

**In Vitro Uptake Inhibition Potency of Rifampicin and Cyclosporine in Monkey Hepatocytes.** Both rifampicin and cyclosporine inhibited OATP-mediated rosuvastatin and pitavastatin uptake in a concentration-dependent manner (Fig. 2). For rosuvastatin, up to 88% inhibition was observed after preincubation with rifampicin (Fig. 2A). The uninhibited uptake of rosuvastatin (12%) was in close agreement with the contribution of passive diffusion (4%) estimated from the uptake kinetic study in monkey hepatocytes. The IC₅₀ of rifampicin obtained after preincubation with buffer (1.14 µM) was reduced by approximately 2-fold after preincubation with rifampicin (0.54 µM) (Table 2). This marginal (2-fold) effect of preincubation on rifampicin potency seen with rosuvastatin reflects similar findings reported for this inhibitor in human OATP1B1- and OATP1B3-transfected human embryonic kidney 293 (HEK293) cells with estradiol-17β-glucuronide as a probe (2- and 3-fold increase in potency; details in Supplemental Table 4). Cyclosporine showed similar extent of maximal inhibition of rosuvastatin uptake in monkey hepatocytes at the highest concentration (Fig. 2C). However, the increase in cyclosporine inhibition potency following preincubation was more pronounced (7-fold shift) (Table 2).

In the case of pitavastatin, maximal 55% inhibition of uptake was observed at the highest rifampicin concentration and after preincubation with inhibitor (Fig. 2B). There was no significant change in the IC₅₀ value after preincubation with rifampicin relative to preincubation with buffer (Table 2). This is in agreement with the data reported in HEK293 cells expressing cynomolgus monkey OATP1B1/1B3 using pitavastatin as a probe, in which preincubation with inhibitor resulted in no significant change in rifampicin IC₅₀ (Supplemental Table 4). Discrepancy between the uninhibited uptake (45%) and contribution of passive diffusion (20%) estimated from uptake kinetic data was apparent in the case of pitavastatin. Cyclosporine also showed incomplete inhibition of pitavastatin uptake (Fig. 2D), but, in contrast to rifampicin, its inhibitory potency increased 3.7-fold following

![Fig. 1. Uptake kinetic profiles of rosuvastatin (A) and pitavastatin (B) measured in cryopreserved cynomolgus monkey hepatocytes plated for 4 hours. Symbols represent the observed total uptake. Solid, dashed, and dotted lines represent the predicted total, active, and passive diffusion-related uptake, respectively based on a mechanistic two-compartment model.](image-url)
preincubation with the inhibitor (Table 2). Additionally, reduction in the steepness of the IC_{50} curves was observed after preincubation in particular with cyclosporine (Fig. 2, C and D). DDI studies with rifampicin SV were also performed to elucidate the possible involvement of NTCP in pitavastatin uptake, which would not be inhibited by rifampicin. The maximum inhibition of pitavastatin uptake increased to 64% after preincubation with rifampicin SV, suggesting some contribution of NTCP in pitavastatin uptake in monkey hepatocytes (Supplemental Fig. 1). No effect of preincubation on rifampicin SV inhibition potency was observed in plated monkey hepatocytes (Table 2). Assuming almost complete inhibition of transporter-mediated uptake of pitavastatin at 1 mM rifampicin SV (Thakare et al., 2017), this uninhibited uptake should represent the contribution of passive diffusion to total cellular uptake, which in this case was higher compared with estimates obtained by mechanistic modeling of kinetic data done over short incubation times. Such incomplete inhibition of uptake for pitavastatin has previously been reported in monkey hepatocytes using rifampicin and cyclosporine (Takahashi et al., 2013) and in human hepatocytes using rifampicin (Pahwa et al., 2017) and may reflect the combination of passive diffusion and involvement of transporter uptake not inhibited by these inhibitors.

### Dose-Dependent Effect of Rifampicin on i.v. and Oral Pharmacokinetics of Rosuvastatin and Pitavastatin in Monkeys

Intravenous pharmacokinetics of stable-labeled rosuvastatin and pitavastatin and the oral pharmacokinetics of rosuvastatin and pitavastatin estimated in plated cynomolgus monkey hepatocytes using a mechanistic two-compartment model.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Km</th>
<th>Vmax</th>
<th>k_{cell}</th>
<th>CL_{diff}</th>
<th>CL_{active}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>3.29</td>
<td>323 ± 44</td>
<td>0.25 ± 0.08</td>
<td>4.27 ± 1.07</td>
<td>98.3</td>
</tr>
<tr>
<td>Pitavastatin</td>
<td>1.99</td>
<td>217 ± 34</td>
<td>0.025 ± 0.009</td>
<td>26.50 ± 2.74</td>
<td>109</td>
</tr>
</tbody>
</table>

**Table 1**

**Fig. 2.** Rifampicin (A and B) and cyclosporine (C and D) concentration-dependent inhibition of rosuvastatin (A–C) and pitavastatin (B–D) in cryopreserved cynomolgus monkey hepatocytes plated for 4 hours. Preincubation with either buffer or inhibitor (rifampicin 0.01–100 μM, cyclosporine 0.01–6 μM) was performed for 1 hour prior to coincubation with the inhibitor and the probe substrates. Data represent mean uptake ± S.D. of at least triplicate measurements.
of cold rosuvastatin and pitavastatin were measured in cynomolgus monkeys after single oral ascending doses of rifampicin (1, 3, 10, and 30 mg), and compared with the vehicle-only dosing (control). The plasma concentration–time profiles and the corresponding pharmacokinetic parameters are shown in Figs. 3–5 and Table 3. Rosuvastatin i.v. clearance, when dosed along with 30 mg/kg rifampicin, decreased to almost 50% of control, whereas there was no significant change in the volume of distribution. This is in agreement with the previously reported marginal decrease in Vdss of i.v. rosuvastatin when given with a lower rifampicin dose in cynomolgus monkey (Chu et al., 2015). Oral AUC and Cmax of rosuvastatin were increased up to 15-fold by the dose in cynomolgus monkey (Chu et al., 2015). Oral AUC and Cmax of pitavastatin were noted with AUC change of up to dependent increase in oral pitavastatin plasma exposure altered significantly by rifampicin. Rifampicin dose-(Takahashi et al., 2013), and, therefore, half-life was not significant decrease in Vdss, consistent with a previous report group). The decrease in i.v. clearance was accompanied by a reduction in i.v. clearance was more prominent (18.0 ml/min/kg highest dose of rifampicin. In the case of pitavastatin, re-duction in i.v. clearance was more prominent (18.0 ml/min/kg versus 4.3 ml/min/kg), and the volume of distribution was reduced from 1.8 (control) to 0.55 l/kg (30 mg/kg rifampicin group). The decrease in i.v. clearance was accompanied by a significant decrease in Vdss, consistent with a previous report (Takahashi et al., 2013), and, therefore, half-life was not altered significantly by rifampicin. Rifampicin dose-dependent increase in oral pitavastatin plasma exposure was noted with AUC change of up to ~19-fold and Cmax increase of up to ~12-fold at the highest dose tested (30 mg/kg) compared with the control group.

The lactone forms of orally administered pitavastatin and rosuvastatin were also measured in plasma samples. The plasma AUC of the lactone forms in the absence and presence of rifampicin and the lactone-acid ratios are reported in Supplemental Tables 5 and 6, respectively. No significant increase in the overall lactone plasma AUC was observed for rosuvastatin (Supplemental Table 5). In the case of pitavastatin lactone, a significant increase in its plasma AUC was observed at 3 mg/kg rifampicin. No clear dose-dependent trend in lactone AUC of both statins was apparent. A significant increase in lactone-acid AUC ratios was observed for rosuvastatin only at 1 mg/kg rifampicin relative to control, whereas no significant changes were seen for pitavastatin at any rifampicin dose (Supplemental Table 6). There was an apparent decrease in lactone-acid AUC ratios for both statins, particularly at the highest rifampicin doses. Previously, a decrease and no change in lactone-acid ratio of pitavastatin (in human) and rosuvastatin (in cynomolgus monkey), respecti-vely, were observed in the presence of rifampicin (Prueksaritanont et al., 2014; Chu et al., 2015). Given that the AUCR of a metabolite to parent reflects both the formation and subsequent elimination of the metabolite (Houston, 1981), such behavior is not unexpected.

**IVIVE of OATP Inhibition.** In vivo inhibitory potency values estimated using unbound Cmax of rifampicin were 0.99 ± 3.8 and 0.22 ± 0.14 μM against CLiv of rosuvastatin and pitavastatin, respectively (Fig. 6; Table 2). In vitro IC50 (0.54 μM), obtained in monkey hepatocytes following rifampicin preincubation and using rosuvastatin as probe sub-strate, described reasonably well rifampicin dose-dependent inhibition of rosuvastatin CLiv. In contrast, in vitro IC50 obtained using pitavastatin as probe substrate did not recover the in vivo inhibition activity of rifampicin against pitavastat-in CLiv. However, in vitro IC50 generated using rosuvastatin described well the in vivo data of pitavastatin. Subsequently, in vitro IC50 value of 0.54 μM and inhibitor unbound Cmax was employed to predict the rifampicin dose-dependent change in AUC of both statins dosed either i.v. or orally (Fig. 7). The prediction bias calculated from the geometric mean fold error was <1.6-fold for both statins, with 78% and 88% of the predicted AUCR values within 2-fold of the observed data for rosuvastatin and pitavastatin, respectively. An apparent underprediction was noted for the rosuvastatin oral AUCR at higher doses of rifampicin. This is likely due to increased oral absorption as a result of inhibition of rosuvastatin intestinal efflux, which was not captured in the current prediction model (see Materials and Methods). To note, exploration of IVIVE using IC50 of 0.42 μM obtained in HEK293 cells expressing cOATP1B1 (lowest value compared with those obtained for other OATPs) and using rosuvastatin as an OATP probe (Shen et al., 2013) resulted in either no or marginal improvement in the prediction bias, highlighting the physiologic relevance of monkey hepatocytes for comparison with the inhibition parameters obtained from the in vivo data in a top-down manner.

The FdFg of rosuvastatin estimated from the i.v. and oral data of each study arm showed on average a 178% (2.8-fold) increase in rosuvastatin FdFg in monkeys receiving 30 mg/kg rifampicin relative to the control arm; this trend was not evident for pitavastatin. Correction of the average predicted AUCR values shown in Fig. 7A by this increase in FdFg reduced the underprediction of the magnitude of rosuvastatin DDI at the highest dose of rifampicin (data not shown).

**Discussion**

In comparison with the increased success of quantitative prediction of CYP-mediated DDIs, the confidence in the successful prediction of OATP-mediated DDIs using in vitro data is still low (Gertz et al., 2013; Prueksaritanont et al., 2014; Jones et al., 2015; Bi et al., 2017; Yoshida et al., 2017).

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**TABLE 2**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Preincubation with Buffer</th>
<th>Preincubation with Inhibitor</th>
<th>In vivo IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>Rifampicin</td>
<td>1.14 ± 0.34**</td>
<td>0.54 ± 0.13**</td>
<td>0.99 ± 3.80</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine</td>
<td>0.72 ± 0.04**</td>
<td>0.10 ± 0.04**</td>
<td>—</td>
</tr>
<tr>
<td>Pitavastatin</td>
<td>Rifampicin</td>
<td>3.89 ± 1.82</td>
<td>2.98 ± 0.78</td>
<td>0.22 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine</td>
<td>0.78 ± 0.14**</td>
<td>0.21 ± 0.06**</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Rifamycin SV</td>
<td>6.17 ± 0.96</td>
<td>4.30 ± 1.28</td>
<td>—</td>
</tr>
</tbody>
</table>

Data represent mean ± S.D. of at least triplicate measurements.

** Significance is denoted by a *p < 0.05, **p < 0.01.
Therefore, the objective of our study was to evaluate the IVIVE of OATP inhibition potential using cynomolgus monkey as a preclinical model and to gain confidence in mechanistic translational approach to predict clinical DDIs mediated by these transporters. Furthermore, it was aimed at improving our understanding of the utility of the cynomolgus monkey as a model for humans to drive evaluation of clearance mechanisms (rate-determining step) and DDI risk. Collective results
Fig. 4. Effect of single ascending oral doses of rifampicin on the i.v. and oral pharmacokinetics of pitavastatin in cynomolgus monkey. Plasma concentration–time profiles of i.v. $^3$H$_4$-pitavastatin (A) and oral cold pitavastatin (B) and the estimated pharmacokinetics (C) are depicted. Pharmacokinetics parameters were estimated, with each monkey serving as its own control. Data represent mean ± S.D. ($n = 4$). One-way analysis of variance with Dunnett’s multiple comparisons test was employed to test significance with *$p < 0.05$; **$p < 0.01$. 
suggested that quantitative OATP1B DDI predictions can be made from in vitro IC\textsubscript{50} measured in primary hepatocytes using multiple probe substrates following preincubation with the inhibitor.

In the current study, both rosuvastatin and pitavastatin showed a high affinity for uptake transport in monkey hepatocytes (Table 1). Rosuvastatin CL\textsubscript{active} was approximately 4-fold greater in the current monkey donor investigated compared with the previous data (Shen et al., 2013), possibly reflecting donor differences in the transporter activity, as the estimated K\textsubscript{m} values were comparable. Although CL\textsubscript{diff} was about 6-fold greater in the donor used in the current study, contribution of the passive process to the overall uptake of rosuvastatin (4.2%) was in agreement with previous reports in monkey hepatocytes (Shen et al., 2013).

In this study, the effect of preincubation on the inhibition of OATP-mediated rosuvastatin and pitavastatin uptake in monkey hepatocytes was investigated using prototypical inhibitors rifampicin and cyclosporine. In contrast to the marginal decrease in rifampicin IC\textsubscript{50} after preincubation, more pronounced increase in cyclosporine potency (up to 7-fold) was seen regardless of the substrate probe used. This clear preincubation effect on cyclosporine inhibition potency demonstrated in this work in monkey hepatocytes is in agreement with previous literature reports on this inhibitor. But the reported magnitude of shift in cyclosporine potency varied between in vitro systems and probes used (Supplementary Table 4), with up to 22- and 23-fold increase in cyclosporine potency noted after preincubation in human and monkey OATP1B1-transfected HEK293 cells, respectively, highlighting that the effect of preincubation on the potency of OATP inhibitors is dependent not only on the substrate used but also on the cellular system investigated.

Incomplete inhibition of uptake was observed for pitavastatin in the current study. As a number of reports demonstrated the contribution of sodium-dependent NTCP to the uptake of pitavastatin and rosuvastatin in human (Bi et al., 2013, 2017) and monkey hepatocytes (Thakare et al., 2017), pitavastatin uptake was further evaluated in the presence of rifamycin SV. Although rifamycin SV increased the maximal inhibition of pitavastatin uptake in monkey hepatocytes, incomplete inhibition of uptake was still evident, highlighting potentially lower transporter activity and/or higher passive contribution in the pooled donor investigated.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+Rifampicin 1 mg/kg</th>
<th>+Rifampicin 3 mg/kg</th>
<th>+Rifampicin 10 mg/kg</th>
<th>+Rifampicin 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pitavastatin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>18.0 ± 4.6</td>
<td>15.0 ± 2.5</td>
<td>7.5 ± 2.0</td>
<td>4.9 ± 1.3</td>
<td>4.3 ± 1.6</td>
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<td>Fold change</td>
<td>0.83</td>
<td>0.42**</td>
<td>0.27**</td>
<td>0.24**</td>
<td></td>
</tr>
<tr>
<td>Vd\textsubscript{a} (l/kg)</td>
<td>1.79 ± 0.72</td>
<td>1.97 ± 0.50</td>
<td>0.47 ± 0.20</td>
<td>0.52 ± 0.16</td>
<td>0.55 ± 0.20</td>
</tr>
<tr>
<td>Fold change</td>
<td>1.10</td>
<td>0.26**</td>
<td>0.29**</td>
<td>0.30**</td>
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<tr>
<td>t\textsubscript{1/2} (h)</td>
<td>5.9 ± 0.9</td>
<td>7.2 ± 1.7</td>
<td>2.9 ± 1.9</td>
<td>3.8 ± 0.7</td>
<td>4.5 ± 0.6</td>
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<tr>
<td>Fold change</td>
<td>1.51</td>
<td>0.50**</td>
<td>0.63</td>
<td>0.76</td>
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<tr>
<td>Oral AUC\textsubscript{last} (ng/h/ml)</td>
<td>299 ± 146</td>
<td>515 ± 417</td>
<td>2690 ± 2710</td>
<td>4070 ± 4357</td>
<td>5508 ± 4579</td>
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<tr>
<td>Fold change</td>
<td>1.72</td>
<td>8.99**</td>
<td>13.60**</td>
<td>19.41**</td>
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<tr>
<td>Oral C\textsubscript{max} (ng/ml)</td>
<td>225 ± 200</td>
<td>520 ± 822</td>
<td>2730 ± 2040</td>
<td>1920 ± 2950</td>
<td>2780 ± 3166</td>
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<td>2.31</td>
<td>12.13**</td>
<td>8.53**</td>
<td>12.36**</td>
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<tr>
<td><strong>Rosuvastatin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>27.5 ± 7.8</td>
<td>23.6 ± 2.4</td>
<td>20.0 ± 2.1</td>
<td>15.6 ± 1.9</td>
<td>13.4 ± 3.7</td>
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<tr>
<td>Fold change</td>
<td>0.86</td>
<td>0.73</td>
<td>0.57**</td>
<td>0.49**</td>
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<tr>
<td>Vd\textsubscript{a} (l/kg)</td>
<td>0.67 ± 0.41</td>
<td>0.61 ± 0.06</td>
<td>0.64 ± 0.16</td>
<td>0.71 ± 0.08</td>
<td>0.79 ± 0.16</td>
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<td>Fold change</td>
<td>0.91</td>
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<tr>
<td>t\textsubscript{1/2} (h)</td>
<td>2.5 ± 0.7</td>
<td>2.5 ± 0.8</td>
<td>2.1 ± 0.3</td>
<td>3.1 ± 0.1</td>
<td>4.8 ± 2.0</td>
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<td>1.23</td>
<td>1.93**</td>
<td></td>
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<td>Oral AUC\textsubscript{last} (ng/h/ml)</td>
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<td>63 ± 28</td>
<td>232 ± 43</td>
<td>467 ± 267</td>
<td>744 ± 512</td>
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<td>Oral C\textsubscript{max} (ng/ml)</td>
<td>15 ± 11</td>
<td>24 ± 29</td>
<td>122 ± 46</td>
<td>150 ± 100</td>
<td>227 ± 169</td>
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<tr>
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<td>7.72**</td>
<td>9.49**</td>
<td>14.37**</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Oral AUC\textsubscript{last} (ng/h/ml)</td>
<td>985 ± 396</td>
<td>11,300 ± 3260</td>
<td>70,100 ± 10,800</td>
<td>240,000 ± 73,900</td>
<td></td>
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<tr>
<td>Fold change</td>
<td>3.8</td>
<td>7.1</td>
<td>8.1</td>
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<td></td>
</tr>
<tr>
<td>Oral C\textsubscript{max} (ng/ml)</td>
<td>176 ± 99</td>
<td>2050 ± 245</td>
<td>7980 ± 2480</td>
<td>24,200 ± 14,100</td>
<td></td>
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<tr>
<td>Fold change</td>
<td>3.8</td>
<td>4.5</td>
<td>4.6</td>
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<td></td>
</tr>
</tbody>
</table>

Data represent mean ± S.D. (N = 4). One-way analysis of variance with Dunnett’s multiple comparisons test was employed to test significance with *p < 0.05; **p < 0.01.
The quantitative translation of interaction noted in monkeys to humans may not be straightforward due to possible species difference in the mechanisms involved in the clearance. However, several recent reports suggested good agreement between cynomolgus monkeys and humans in the magnitude of DDIs with rifampicin as an OATP inhibitor (Shen et al., 2013; Watanabe et al., 2015). In this study, rifampicin showed a dose-dependent effect with no further change in statins pharmacokinetics between rifampicin doses of 10 and 30 mg/kg in monkeys. The unbound C\text{max} of rifampicin achieved (∼2–7 μM) at these doses is comparable to the unbound C\text{max} in humans following single 600 mg dose (Varma et al., 2012; Prueksaritanont et al., 2014; Yoshikado et al., 2017). However, the magnitude of change in the oral AUC for both statins observed in the cynomolgus monkeys (∼15 to 20-fold, Table 3) is much higher than noted in humans (AUCR ~5) (Prueksaritanont et al., 2014). In contrast, an earlier study showed ∼3-fold increase in oral rosuvastatin AUC in cynomolgus monkey with rifampicin dose of 15 mg/kg orally (Shen et al., 2013). Although the reasons for such a difference are not apparent, a relatively smaller magnitude of rosuvastatin DDI in humans may be attributed to the contribution of OATP2B1 to its hepatic uptake, which is not affected by rifampicin at in vivo relevant plasma concentrations (OATP2B1 in vitro IC\text{50} = 0.67 μM). However, the contribution of OATP2B1 to rosuvastatin hepatic uptake in monkey is likely to be minor because of its relatively low expression in cynomolgus liver (Wang et al., 2015). In addition to OATPs, both rosuvastatin and pitavastatin are substrates of efflux transporters, such as multidrug resistance protein

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**Fig. 6.** IVIVE of hepatic uptake inhibition by rifampicin in cynomolgus monkey. Plots depict rifampicin plasma concentration-dependent inhibition of i.v. clearance of rosuvastatin (A) and pitavastatin (B). Data were fitted to eq. 7 to estimate in vivo IC\text{50} with each statin (red curves with shaded area: 95% confidence interval). Additionally, change in i.v. clearance was predicted using in vitro IC\text{50} obtained in monkey hepatocytes following preincubation with rifampicin with rosuvastatin as a probe (green curve). Vertical lines represent in vivo and in vitro IC\text{50} values (Table 2).

**Fig. 7.** Predicted versus observed interactions between rifampicin and rosuvastatin (A) or pitavastatin (B), when administered with single ascending oral doses of rifampicin. Change in exposure (AUCR) of i.v. (open symbols) and oral (closed symbols) statins was predicted based on eqs. 4–7 using the measured unbound C\text{max} of rifampicin and in vitro IC\text{50} of 0.54 μM. Each monkey served as its own control in calculating the AUCR. Solid and dotted lines represent the line of unity and 2-fold error, respectively.
2 (MRP2) and breast cancer resistance protein (BCRP) (Prueksaritanont et al., 2014; Lee et al., 2015). As rifampicin is also indicated as a substrate of OATP1B1s (Yamaguchi et al., 2011), its inhibitory effect on the biliary efflux of rosuvastatin and pitavastatin was considered. Previously, Chu et al. (2015) showed relatively weak inhibition of cynomolgus MRP2 (cMRP2) by rifampicin in HEK293 cells (IC_{50} of 118 μM). Assuming the reported unbound liver-to-plasma concentration ratio (K_{pu}) of 3.3 for rifampicin in humans (Chu et al., 2015) is comparable to that in cynomolgus monkeys, the IC_{50} for cMRP2 is much higher than the estimated rifampicin-unbound liver concentrations (approximately 23 μM at the highest rifampicin dose used), suggesting that the increase in plasma exposure of both statins is unlikely to be caused by MRP2 inhibition. In case of potential MRP2 inhibition, expectation is that liver AUC will change, consistent with an understanding of the rate-determining processes that affect hepatic exposure of these drugs (Tsamandouras et al., 2015).

The increase in F_{r/F_c} of rosuvastatin seen in this study is most likely attributed to the effect of rifampicin on intestinal BCRP. Rosuvastatin absorption in humans is approximately 50% of the oral dose (Martin et al., 2003), whereas only ~20% was noted in monkeys (control arm). This difference in F_{r/F_c} values suggests species differences in the expression of the intestinal transporters, including BCRP. This is supported by previous reports of generally greater mRNA expression of intestinal efflux transporters in cynomolgus monkeys relative to humans (60-fold difference in the case of BCRP) (Takahashi et al., 2008). Although we cannot assess the differential effects of intestinal OATP2B1 uptake versus BCRP efflux definitively, increased absorption following rifampicin administration implies that BCRP efflux plays a prominent role in the oral absorption of rosuvastatin in monkeys. In contrast, pitavastatin is a highly permeable drug, and the F_{r/F_c} (~0.7 in control arm) is not expected to be limited by intestinal efflux (El-Kattan et al., 2016). Taken together, the current monkey study suggests potential contribution of intestinal efflux inhibition to the rifampicin-rosuvastatin DDIs.

The rich dataset of rifampicin dose-dependent effect on two statins simultaneously dosed i.v. and orally was leveraged to investigate the IVIVE of OATP1B1-mediated DDIs. The in vivo IC_{50} values, estimated using i.v. clearance of rosuvastatin/pitavastatin and unbound C_{max} of rifampicin, were within 2.5-fold of the in vitro IC_{50} (0.54 μM) obtained following preincubation with rifampicin and using rosuvastatin as probe substrate in primary monkey hepatocytes. This in vitro IC_{50} predicted the AUCR of both statins after i.v. and oral dosing with high accuracy (minimum 78% within 2-fold of the observed data), when employing the static mechanistic model (eq. 7). In contrast, the in vitro IC_{50} obtained under all other experimental conditions (Table 2) considerably underestimated the fold change in the AUC of statins investigated. Consistent with the IVIVE noted in this work in monkeys, Varma et al. (2014) demonstrated that similar inhibition potency (0.5 μM), obtained using OATP1B1-transfected cells, correctly predicted rifampicin clinical DDIs when using a static mechanistic model (>85% within 2-fold of observed AUCRs, n = 22). Overall, the current monkey study validates mechanistic translational framework to predict OATP1B1-mediated DDIs in humans.

Substrate-dependent inhibition was argued as a potential cause for concern in prospective prediction of OATP1B1-mediated interactions (Noé et al., 2007; Izumi et al., 2013; Zamek-Gliszczynski et al., 2013). We report almost 6-fold difference in rifampicin unbound IC_{50} measured with preincubation when using rosuvastatin (0.54 μM) versus pitavastatin (3.0 μM) (Table 2). Interestingly, the change in i.v. clearance and oral exposure of both rosuvastatin and pitavastatin is recovered well only by the rifampicin in vitro IC_{50} measured against rosuvastatin (Figs. 6 and 7). Based on these findings, it can be inferred that: 1) substrate-dependent inhibitory potency noted in vitro may not translate to differences in vivo, 2) IC_{50} measured following preincubation with inhibitor is needed for quantitative prediction of OATP-mediated DDIs, and 3) in vitro inhibition studies should consider more than one probe substrate, and the most potent measurement should be employed for reliable prospective predictions. Of note, plasma coproporphyrins (I and III) have recently been described as selective and sensitive OATP1B1 biomarkers in both cynomolgus monkeys and humans (Shen et al., 2016; Barnett et al., 2017). Therefore, the coordinated use of the cynomolgus monkey (prehuman dosing), with measurements of changes in biomarker exposure in Phase 1, could be leveraged to quickly discharge OATP1B DDI risk. Given that current regulatory agency-driven OATP1B DDI risk analyses are conservative and present a relatively high false-positive rate (~30%) (Vaidyanathan et al., 2016), consideration of such approaches is warranted.

In summary, our studies using the cynomolgus monkey as a preclinical model demonstrated a rational translation of in vitro inhibition potency in predicting OATP1B DDIs. Rifampicin in vitro inhibition potency measured using monkey hepatocytes was influenced by a preincubation step and/or the probe substrate employed. However, in vivo IC_{50} values obtained from the change in rosuvastatin or pitavastatin i.v. clearance as a function of rifampicin unbound C_{max} were not statistically different. Additionally, the most potent IC_{50} obtained under preincubation conditions provided good quantitative predictions of fold changes in plasma AUC for both statins following oral administration. Collectively, these findings suggest the need for employing multiple probe substrates and a preincubation step when conducting in vitro inhibition studies. Finally, this study emphasizes further the utility of cynomolgus monkey as a preclinical model in supporting the early assessment of OATP-mediated DDI risk.

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Authorship Contributions
Participated in research design: Ufuk, Kosa, Gao, Bi, Rodrigues, Tremaine, Varma, Houston, Galetin.
Conducted experiments: Ufuk, Kosa, Gao, Bi, Modi, Gates.
Contributed new reagents or analytic tools: Gao.
Performed data analysis: Ufuk, Kosa, Varma, Houston, Galetin.
Wrote or contributed to the writing of the manuscript: Ufuk, Kosa, Gao, Modi, Gates, Rodrigues, Tremaine, Varma, Houston, Galetin.

References