Quantitative Prediction of Transporter- and Enzyme-Mediated Clinical Drug-Drug Interactions of Organic Anion-Transporting Polypeptide 1B1 Substrates Using a Mechanistic Net-Effect Model

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ABSTRACT
Quantitative prediction of complex drug-drug interactions (DDIs) involving hepatic transporters and cytochromes P450 (P450s) is challenging. We evaluated the extent of DDIs of nine victim drugs—which are substrates to organic anion-transporting polypeptide 1B1 and undergo P450 metabolism or biliary elimination—caused by five perpetrator drugs, using in vitro data and the proposed extended net-effect model. Hepatobiliary transport and metabolic clearance estimates were obtained from in vitro studies. Of the total of 62 clinical interaction combinations assessed using the net-effect model, 58 (94%) could be predicted within a 2-fold error, with few false-negative predictions. Model predictive performance improved significantly when in vitro active uptake clearance was corrected to recover in vivo clearance. The basic R-value model yielded only 63% predictions within 2-fold error. This study demonstrates that the interactions involving transporter-enzyme interplay need to be mechanistically assessed for quantitative rationalization and prospective prediction.

Introduction
The ability to quantitatively predict drug-drug interactions (DDIs) early in drug development is essential to minimize unexpected clinical study readouts and manage the adverse risks associated with drug interactions. Confidence in the prediction of DDIs for drugs eliminated via cytochrome P450 (P450) enzymes is generally high (Obach et al., 2006; Fahmi et al., 2008). However, despite tremendous strides in several areas of drug transporters, reliable tools for quantitative prediction of transporter-based DDIs are not well established. In addition, significant challenges arise in the evaluation and/or prediction of complex drug interactions caused by perpetrator drugs and metabolites that affect multiple (transporter- and enzyme-mediated) disposition processes (Hinton et al., 2008; Yoshida et al., 2012).

In liver, organic anion-transporting polypeptides (OATPs) OATP1B1, OATP1B3, and OATP2B1 are expressed on the sinusoidal membrane of hepatocytes and facilitate uptake of many clinically important anionic drugs, including 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) (Shitara and Sugiyama, 2006; Kallikoski and Niemi, 2009; Giacomini et al., 2010; Fenner et al., 2012). Indeed, clinically relevant DDIs are attributed to the inhibition of transport mediated by members of the OATP family (Shitara et al., 2006). Furthermore, polymorphisms in SLC01B1 (encoding OATP1B1) were demonstrated with altered transporter activity leading to significant change in systemic exposure of statins, which could regulate relative peripheral tissue exposure and the risk of muscle toxicity (Nishizato et al., 2003; Niemi et al., 2005; Link et al., 2008; Ieiri et al., 2009). Although hepatic uptake was suggested to be the rate-determining process in the systemic clearance of several OATP transporters, substrates including statins, enzymatic metabolism, and/or biliary efflux also contribute to the systemic clearance and elimination from the body (Shitara et al., 2006; Watanabe et al., 2009; Maeda et al., 2011; Varma et al., 2012, 2013a). For instance, atorvastatin is majorly metabolized by the CYP3A4, whereas repaglinide and cerivastatin are metabolized by CYP2C8 and CYP3A4. Canalicul efflux transporters, breast cancer–resistant protein (BCRP), and multidrug resistance protein 2 (MRP2) are identified to be driving biliary elimination of rosuvastatin and pravastatin, respectively.

A conservative assessment of OATP-mediated DDIs can be made by a simplified static R-value model, which assumes active uptake solely contributing to clearance of the substrate victim drug (Giacomini et al., 2010; European Medicines...
Materials and Methods

Clinical DDI and In Vitro Data Collection. Clinical DDI data combinations involving nine victim drugs and five perpetrator drugs were primarily extracted from the University of Washington metabolism and transporter drug interaction database (www.druginteractioninfo.org). Additional exhaustive literature search was conducted to enrich the clinical DDI dataset. In vitro interaction potency data were collected from scientific literature. Corresponding references were cited in Supplemental Material.

Transport Studies Using Sandwich Culture Human Hepatocytes. Cryopreserved human hepatocytes lot BD310 (male donor) and lot 109 (male donor) were purchased from BD Biosciences (Woburn, MA), and lot Hu4168 (female donor) was obtained from Life Technologies (Carlsbad, CA). Drug substances were purchased from Sequoia Research Products (Pangbourne, UK) and Sigma-Aldrich (St. Louis, MO). All other chemicals were purchased from Sigma-Aldrich. The sandwich culture human hepatocyte (SCHH) methodology was described previously (Bi et al., 2006). Briefly, cryopreserved hepatocytes were thawed and plated with cell density of 0.75 × 10^6 cells/ml. The plates were overlaid with 0.25 mg/ml matrigel on the second day, and the cultures were maintained. On day 5, to determine the rates of uptake and passive diffusion, the cells were preincubated with or without 100 μM rifamycin SV, and to determine biliary clearance the cells were preincubated with or without Ca^2+ Hanks’ balanced salt solution buffer for 10 minutes. The reactions were initiated by addition of 1 μM victim drug and were terminated at predetermined time points by washing the cells three times with ice-cold Hanks’ balanced salt solution. Cells were then lysed with 100% methanol containing internal standard, and the samples were analyzed by liquid chromatography–tandem mass spectrometry (Supplemental Methods).

Static Mechanistic Extended Net-Effect Model. The area under the plasma concentration–time curve (AUC) ratio (AUCR) of oral victim drug in the presence (AUC_{po}) and absence (AUC_{po}) of perpetrator can be described by the following equations:

\[
AUCR = \frac{AUC_{po} \cdot \frac{F_a}{F_g} \cdot \frac{F_h}{F_g} \cdot (CL_{u,b} + CL_u)}{AUC_{po} \cdot \frac{F_a}{F_g} \cdot \frac{F_h}{F_g} \cdot (CL_{u,b} + CL_u)}
\]

(1)

\[
F_h = 1 - \frac{CL_u}{Q_{ch}}
\]

(2)

\[
CL_u = \frac{Q_{ch} \cdot f_{u,b} \cdot CL_{int,h}}{Q_{ch} + f_{u,b} \cdot CL_{int,h}}
\]

(3)

where \(F_a\), \(F_g\), and \(F_h\) represent the fraction of drug absorbed, fraction of drug escaping gut-wall extraction, and fraction of drug escaping hepatic extraction, respectively. \(F_{u,b}\) and \(F_{u,b}\) are corresponding parameters in the presence of perpetrator. Hepatic blood clearance (\(CL_u\)), renal clearance (\(CL_r\)), \(CL_{int,h}\), and \(CL_{int,b}\) represent hepatic and renal blood clearance in the absence and presence of the perpetrator, respectively. \(CL_{int,h}\) is intrinsic hepatic clearance, \(CL_{int,b}\) is fraction unbound in blood, and Q is hepatic blood flow [20.7 ml/min per kg (Kato et al., 2003)]. Assuming no or negligible tubular re-absorption, renal clearance can be expressed as a function of glomerular filtration rate (GFR; 1.78 ml/min per kg) and active secretion (\(CL_{a,sec}\)) as:

\[
CL_{a,sec} = f_{a,b} \cdot GFR + CL_{a,sec}
\]

(5)

\[
CL_{int,h} = \frac{SF_{active} \cdot PS_{active} + PS_{pd}}{PS_{pd} + \sum CL_{int,p450} + CL_{int,bile}}
\]

(4)

where SF_{active} and PS_{active} are sinusoidal active uptake clearance and passive diffusion, respectively. Active uptake was assumed to be primarily OATP1B1-mediated transport. CL_{int,bile} is biliary intrinsic clearance. \(CL_{int,p450}\) represents the sum of intrinsic metabolic clearances by individual P450s and can also be expressed as follows:

\[
CL_{int,mixed} = f_{u,p450} \cdot CL_{int,p450} \cdot (1 - f_{u,p450} \cdot x) + \sum CL_{int,mixed} \cdot (1 - 1 - f_{u,p450} \cdot x), \text{where } CL_{int,mixed} \text{ is the total intrinsic metabolic clearance, } SF_{active} \text{ represents empirical scaling factor for active uptake estimated by matching the in vitro } CL_{int,h}\text{ (eq. 5) to the in vivo } CL_{int,h}\text{, obtained from intravenous pharmacokinetics (eq. 17). The in vitro intrinsic values were scaled assuming the following: } 118 \times 10^6 \text{ hepatocytes } g^{-1} \text{ liver, } 39.8 \text{ mg microsomal protein } g^{-1} \text{ liver, and } 24.5 \text{ g liver kg }^{-1} \text{ body weight (Varma et al., 2013b).}

In the presence of perpetrator, the expected net effect of reversible inhibition, time-dependent inhibition, and metabolic induction can be illustrated by the following:

\[
CL_{int,h} = \frac{SF_{active} \cdot PS_{active}}{RI_{OATP}} \cdot \left(\frac{CL_{int,p450} + CL_{int,bile}}{PS_{pd} + \sum CL_{int,p450} + CL_{int,bile}}\right)
\]

(6)

where \(RI_{OATP}\) is the competitive inhibition term (eq. 7), \(TDI_{h,p450}\) is the time-dependent inhibition term (eq. 8), and \(IND_{h,p450}\) is the hepatic induction term (eq. 9) for the interactions associated with the particular P450 isoform. These interaction terms will be reduced to one for enzymes not affected by the perpetrator and its metabolite.

RI_{OATP} is the competitive inhibition term (eq. 7) for active hepatic uptake, and RI_{efflux} is the competitive inhibition term (eq. 7) for biliary efflux transport (Fahmi et al., 2008; Giacomini et al., 2010; US FDA, 2012; Barton et al., 2013).
the change of the fraction of drug escaping intestinal extraction in the blood flow, respectively. Consistent with the earlier reports, absorption rate constant, fraction unbound in the gut, and enterocytic

\[
\text{CL}_{\text{int},\text{h}} = \frac{\text{CL}_h}{f_{\text{u},\text{h}} \left(1 - \frac{\text{CL}_\text{deg}}{\text{CL}_h}\right)}
\]  

(17)

where \(\text{CL}_\text{deg} = (\text{CL}_p - \text{CL}_\text{efflux})\) is the hepatic blood clearance obtained from intravenous total plasma clearance corrected for renal clearance and blood-to-plasma ratio (\(R_b\)).

\(K_{p,u}\) represents the unbound concentration in liver relative to the unbound concentration in plasma at steady state, given as follows (Liu and Pang, 2005; Shitara et al., 2006; Barton et al., 2013):

\[
K_{p,u} = \frac{\text{SF}_{\text{active}} \times \text{PS}_{\text{active}} + \text{PS}_{\text{pd}}}{\text{PS}_{\text{pd}} + \sum \text{CL}_{\text{int},\text{P450}} + \text{CL}_{\text{int},\text{bile}}}
\]  

(18)

Model Predictability. All calculations were made using Microsoft Office Excel 2007. Prediction bias and precision were also assessed with root mean square error (RMSE) (eq. 19) and average fold error (AFE) (eq. 20).

\[
\text{RMSE} = \sqrt{\frac{\sum (\text{Predicted} - \text{Observed})^2}{N}}
\]  

(19)

\[
\text{AFE} = 10 \times \frac{1}{N} \sum \left| \frac{\text{Predicted}}{\text{Observed}} - 1 \right|
\]  

(20)

\(N\) is the number of observations.

**Results**

**In Vitro Hepatobiliary Transport of OATP Substrates.** Active and passive hepatobiliary transport clearance of 10 OATP1B1 substrates was determined in vitro using SCHH from three cyropreserved hepatocyte lots. All drugs showed active uptake—wherein rifampicin is (Paine et al., 2006), the change of the fraction of drug escaping intestinal extraction in the presence of perpetrator can be defined by eq. 10 (Fahmi et al., 2008).

\[
\text{F}_g' = \frac{1}{1 + \frac{\text{I}_{\text{u},\text{in}}}{\text{K}_i}}
\]  

(10)

where, \(\text{RI}_p\), \(\text{TDI}_p\), and \(\text{IND}_p\) are the reversible inhibition (eq. 11), time-dependent inhibition (eq. 12), and induction (eq. 13) terms for CYP3A4-mediated gut metabolism.

\[
\text{RI}_p = 1 + \frac{\text{I}_{\text{max}}}{\text{K}_i}
\]  

(11)

\[
\text{TDI}_p = \frac{\text{K}_{\text{deg},p} + \frac{\text{I}_{\text{max}}}{\text{K}_i}}{\text{K}_{\text{deg},p}}
\]  

(12)

\[
\text{IND}_p = \frac{1}{1 + \frac{\text{I}_{\text{max}}}{\text{K}_i}}
\]  

(13)

\(I_{\text{u},\text{gut}}\), the free intestinal concentration of the perpetrator, was estimated by eq. 15.

\[
\text{I}_{\text{u},\text{max,in}} = f_{\text{u},\text{h}} \left(\text{I}_{\text{max}} + \frac{\text{Dose} \times K_p \times F_p}{Q_h}\right)
\]  

(14)

\[
\text{I}_{\text{u},\text{gut}} = \frac{\text{Dose} \times K_p \times F_p}{Q_h}
\]  

(15)

Dose, \(I_{\text{max},b}\), \(K_p\), \(f_{\text{u},\text{gut}}\), and \(Q_{\text{gut}}\) [248 ml/min (Fahmi et al., 2008)] represent total dose given orally, maximum total blood concentration, absorption rate constant, fraction unbound in the gut, and enterocytic blood flow, respectively. Consistent with the earlier reports, \(K_{\text{deg}}\) was assumed to be 0.019 h\(^{-1}\) for hepatic CYP3A4 and CYPC28 and 0.029 h\(^{-1}\) for intestinal CYP3A4 (Fahmi et al., 2008; Lai et al., 2009).

Change in active renal secretion (\(\text{CL}_{\text{f,sec}}\)) caused by inhibition of OAT3 by gemfibrozil is described by a basic model (Feng et al., 2013).

\[
\text{CL}_{\text{f,sec}} = \frac{\text{CL}_{\text{f,sec}}}{1 + \frac{\text{I}_{\text{max}} \times f_{\text{u},\text{h}}}{\text{K}_{\text{max}}}}
\]  

(16)

In vivo \(\text{CL}_{\text{int},\text{h}}\) was calculated using the well stirred liver model (Pang and Rowland, 1977).
maximum perpetrator concentration in the gut ($I_{u,gut}$) and the inlet to liver ($I_{u,max,in}$).

Interactions with cyclosporine are evaluated assuming reversible inhibition of OATP1B1, MRP2, BCRP, and CYP3A4 (Tables 1 and 2). Predicted AUCRs were within 2-fold of the observed mean value for 10 of 12 (83%) cases, when using drug-specific SFactive (Fig. 2A; Supplemental Table 1). Precision and bias analysis of the cyclosporine DDIs yielded RMSE and AFE of 4.3 and 1.6, respectively (Table 3). Alternatively, R-value (Giacomini et al., 2010; European Medicines Agency, 2012; US FDA, 2012) was calculated considering only OATP1B1 inhibition and compared with the predictions of current model. Although the calculated R-value and the predicted AUCRs were similar in majority of the interactions with cyclosporine, R-value overpredicted (>2-fold error) exposure change in 25% of cases (Fig. 2A).

Interactions with gemfibrozil were evaluated, wherein gemfibrozil was assumed to be reversibly inhibit OATP1B1, whereas its major circulating metabolite, gemfibrozil 1-Oβ-glucuronide, cause reversible inhibition of OATP1B1 and time-dependent inhibition of CYP2C8. Additionally, inhibition of OAT3-mediated renal secretion of pravastatin and rosuvastatin was considered. Model predictions are within 2-fold error for 93% (14 of 15) cases, with RMSE and AFE of 2.4 and 1.4, respectively (Fig. 2B, Table 3). In contrast, R-value predicted only 46% interactions within 2-fold.

### TABLE 1
Summary of input parameters for victim drugs used in the mechanistic model-based predictions of transporter- and cytochrome P450–based drug-drug interactions

<table>
<thead>
<tr>
<th>Victim Drug</th>
<th>Observed Plasma CL (ml/min per kg)</th>
<th>Plasma CL (ml/min per kg)</th>
<th>Fg</th>
<th>Plasma fa</th>
<th>Rb</th>
<th>f u,max,in</th>
<th>K p,vn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>7.8</td>
<td>0</td>
<td>0.60</td>
<td>0.024</td>
<td>0.61</td>
<td>853</td>
<td>12.4</td>
</tr>
<tr>
<td>Bosentan</td>
<td>2.1</td>
<td>0</td>
<td>0.98</td>
<td>0.037</td>
<td>0.66</td>
<td>67</td>
<td>35.5</td>
</tr>
<tr>
<td>Cerivastatin</td>
<td>2.9</td>
<td>0</td>
<td>0.74</td>
<td>0.014</td>
<td>0.76</td>
<td>254</td>
<td>16.8</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>8.7</td>
<td>0</td>
<td>1.00</td>
<td>0.008</td>
<td>0.57</td>
<td>4141</td>
<td>30.7</td>
</tr>
<tr>
<td>Glyburide</td>
<td>0.8</td>
<td>0</td>
<td>0.97</td>
<td>0.021</td>
<td>0.58</td>
<td>42</td>
<td>15.8</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>5.7</td>
<td>0</td>
<td>1.00</td>
<td>0.025</td>
<td>0.58</td>
<td>434</td>
<td>34.8</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>13.5</td>
<td>6.3</td>
<td>1.00</td>
<td>0.47</td>
<td>0.56</td>
<td>70</td>
<td>1.4</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>11.7</td>
<td>3.3</td>
<td>1.00</td>
<td>0.12</td>
<td>0.69</td>
<td>171</td>
<td>9.1</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>7.8</td>
<td>0</td>
<td>0.94</td>
<td>0.015</td>
<td>0.62</td>
<td>1326</td>
<td>35.5</td>
</tr>
<tr>
<td>Valsartan</td>
<td>0.5</td>
<td>0.15</td>
<td>1.00</td>
<td>0.01</td>
<td>0.55</td>
<td>35</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**CL,** clearance; **CLu,hi,** biliary intrinsic clearance; **CLu,lo,** intrinsic hepatic clearance; **CLr,** renal clearance; **Emax,** maximum fold induction; **Fg,** fraction of drug absorbed; **Fdg,** fraction of drug escaping gut-wall extraction; **Fuc,** fraction unbound; **f u,gut,** fraction unbound in the gut; **K p,** absorption rate constant; **K i,** inhibition constant; **K u,lo,** liver-to-plasma unbound concentration ratio; **MRP2,** multidrug resistance protein 2; **OATP,** organic anion-transporting polypeptide; **PSactive,** sinusoidal active uptake clearance; **PSpd,** passive diffusion; **Rb,** blood-to-plasma ratio; **SFactive,** active uptake scaling factor.

*Intravenous clearance, except for pitavastatin, in which case clearance was obtained by correcting the oral clearance with the estimated oral bioavailability (taken from Yoshida et al., 2012).*

*In vitro data was calculated using eq. 17.

*In vitro mean data from independent sandwich culture human hepatocyte studies using three different hepatocyte lots (see Materials and Methods). *

*In vitro data based on independent sandwich culture human liver microsomes or recombinant P450s (see Materials and Methods). No significant metabolism was observed for pravastatin, rosuvastatin, and valsartan.

*Values corrected for microsomal binding.

*Remaining fraction metabolism is associated with CYP2C8.

*Remaining fraction metabolism is associated with CYP2C9.

*Biliary clearance was assumed to be mediated by BCRP (pitavastatin and rosvastatin) and MRP2 (pravastatin).
Fig. 1. In vitro–in vivo extrapolation of hepatic intrinsic clearance of 10 OATP1B1 substrate drugs. In vitro hepatic intrinsic clearance was calculated using extended clearance equation assuming SFactive of unity (A) and geometric mean value (B). In vitro intrinsic clearance was significantly underpredicted when the SCHH and human liver microsome data were directly adopted (SFactive of unity) in extended clearance equation. After applying geometric mean SFactive of 10.6, 60% drugs are within 2-fold and 70% within 3-fold of the observed values. Data points represent SCHH data from hepatocyte lot BDS10 (△), lot 109 (○), lot Hu4168 (●), and the mean of three lots (–). Diagonal solid and dashed lines represent unity and 2-fold error, respectively. A, atorvastatin; B, bosentan; C, cerivastatin; F, fluvastatin; G, glyburide; P, pitavastatin; Pr, pravastatin; R, rosuvastatin; Re, repaglinide; V, valsartan.

Rifampicin DDIs with victim drugs were well predicted (∼95% within 2-fold), assuming OATP1B1 inhibition and/or CYP3A4 induction, on case basis (Fig. 2C; Supplemental Table 3). For instance, in DDI studies involving single concomitant dosing of victim drug and rifampicin, only OATP1B1 inhibition by rifampicin was considered. On the contrary, when victim drug was dosed within 12.5 hours after the last dose of rifampicin chronic pretreatment (5 or 7 days), both OATP1B1 inhibition and CYP3A4 induction activity were assumed simultaneously; however, when dosed after 12.5 hours, only CYP3A4 induction was considered (Varma et al., 2013b). Incorporation of change in Fp, caused by CYP3A4 induction, significantly improved DDI predictions (Fig. 2C). No considerable change in the predictions of rifampicin-based DDIs was noted following sensitivity analysis to evaluate the effect of d-factor (eq. 9) (Supplemental Fig. 1).

All predicted AUCRs were within 2-fold for DDIs involving potent CYP3A4 inhibitors, itraconazole, and clarithromycin (Fig. 2D; Supplemental Table 4). In case of interactions with itraconazole, reversible inhibition of CYP3A4 by both parent and metabolite (4-hydroxyl itraconazole) was considered. In contrast, clarithromycin showed moderate reversible inhibition of OATP1B1 and potent TDI of CYP3A4. RMSE and AFE were low (0.5 and 1.3, respectively), although five false-negative predictions were noted with these perpetrator drugs (Table 3).

**Effect of SFactive on AUCR Prediction.** A comparison of the AUCR predictions assuming SFactive of unity (no correction for in vitro–in vivo disconnect in Clint,h), geometric mean of 10 substrate drugs (SFactive = 10.6), and drug-specific value showed that the model predictability was lowest when the in vitro active rate was not corrected (Fig. 3; Table 3). Correspondingly, the model-predictive performance was 63, 86, and 94% (within 2-fold error), suggesting that correcting the active uptake rate not only better recovers the in vivo clearance of the victim drugs, but also quantitatively explains their DDIs.

**Predicted Change in Free Liver-to-Plasma Ratio (Kp,uu/Kp,uu).** To assess the change in free liver concentrations due to DDIs, free liver-to-plasma concentration ratio of the victim drug was calculated in the absence (Kp,uu) and presence (Kp,uu) of perpetrator drug. The ratio of ratios (Kp,uu/Kp,uu) indicated that inhibition of OATP1B1 by cyclosporine could reduce the Kp,uu by ∼75–90% (Fig. 4). Gemfibrozil increased Kp,uu at low doses; however, it reduced the Kp,uu by as much as ∼75% at high doses. The simultaneous inhibition of OATP1B1-mediated uptake and induction of CYP3A4 activity following multiple-dose rifampicin treatment caused largest changes in Kp,uu (∼98% reduction). In contrast, single-dose rifampicin increased plasma AUC while decreasing Kp,uu. On the basis of the observed AUCRs and the predicted Kp,uu/Kp,uu ratios, the interactions were classified into four classes, as follows: class A interactions, increased systemic exposure and Kp,uu/Kp,uu; class B interactions, increased systemic exposure and decreased Kp,uu/Kp,uu; class C interactions, decreased systemic exposure and Kp,uu/Kp,uu; and class D interactions, decreased systemic exposure and increased Kp,uu/Kp,uu.

**Discussion**

This study demonstrated that the DDIs of OATP1B1 substrates involving transporter-enzyme interplay can be quantitatively predicted using the proposed static extended net-effect model. The predictive evaluation not only considers multiple mechanisms of interaction of the perpetrator drug (and metabolite) simultaneously, but also captures the individual components of the overall clearance of the victim drug to evaluate transporter-mediated and complex DDI situations. For a set of nine OATP1B1 substrates, 94% of the 62 combinations of clinical interactions with perpetrator drugs that affect OATP1B1 and/or P450 activity were predicted within 2-fold error. Furthermore, the precision and bias analysis of whole dataset (RMSE and AFE of 2.4 and 1.4, respectively) suggested improved accuracy of the model compared with conventional models (e.g., R-value).

The current SCHH data showed significant active transport for all victim drugs, which were also confirmed to be OATP1B1.
substrates based on in vitro cellular uptake studies (data not shown). The hepatic clearance of the OATP1B1 substrates was considerably underpredicted, when estimated using in vitro metabolic/biliary intrinsic clearance alone, suggesting a key role of hepatic uptake in their disposition. The rate-determining role of the uptake transport for some of these drugs was also demonstrated in clinical studies and PBPK-based assessments (Watanabe et al., 2009; Maeda et al., 2011; Jones et al., 2012; Varma et al., 2013a, 2014; Jamei et al., 2014). However, overall hepatic intrinsic clearance (calculated

### Table 3

<table>
<thead>
<tr>
<th>DDI Drugs</th>
<th>N</th>
<th>R-Value</th>
<th>SF&lt;sub&gt;active&lt;/sub&gt; = 1</th>
<th>SF&lt;sub&gt;active&lt;/sub&gt; = Geometric Mean (10.6)</th>
<th>SF&lt;sub&gt;active&lt;/sub&gt; = Drug-Specific</th>
</tr>
</thead>
</table>
| **Observed versus predicted change in systemic exposure of the OATP1B1 substrate drugs when administered with (A) cyclosporine, (B) gemfibrozil, (C) rifampicin, and (D) itraconazole or clarithromycin. Data points represent AUCR (♦), R-value (♦), and the change in overall hepatic intrinsic clearance (−). Diagonal solid and dashed lines represent unity and 2-fold error, respectively.** Another role of hepatic uptake in their disposition. The rate-determining role of the uptake transport for some of these drugs was also demonstrated in clinical studies and PBPK-based assessments (Watanabe et al., 2009; Maeda et al., 2011; Jones et al., 2012; Varma et al., 2013a, 2014; Jamei et al., 2014). However, overall hepatic intrinsic clearance (calculated

| DDI Drugs                  | R-Value | % within 2-Fold | FN | RMSE | AFE | R-Value | % within 2-Fold | FN | RMSE | AFE | R-Value | % within 2-Fold | FN | RMSE | AFE | R-Value | % within 2-Fold | FN | RMSE | AFE | R-Value | % within 2-Fold | FN | RMSE | AFE | R-Value | % within 2-Fold |
|----------------------------|---------|-----------------|----|------|-----|---------|-----------------|----|------|-----|---------|-----------------|----|------|-----|---------|-----------------|----|------|-----|---------|-----------------|----|------|-----|---------|-----------------|----|------|-----|---------|-----------------|----|------|-----|---------|-----------------|
| Cyclosporine               | 12      | 4.6             | 1.6| 75    | 2   | 6.2     | 2.9             | 42 | 0    | 1.8 | 67  | 0    | 4.3  | 1.6             | 83 | 0    | 1.8 | 67  | 0    | 4.3  | 1.6             | 83 | 0    | 1.8 | 67  | 0    | 4.3  | 1.6             | 83 |
| Gemfibrozil                | 15      | 4.8             | 2.2| 46    | 0   | 3.3     | 1.8             | 60 | 0    | 1.4 | 87  | 0    | 2.4  | 1.4             | 93 | 0    | 1.4 | 87  | 0    | 2.4  | 1.4             | 93 | 0    | 1.4 | 87  | 0    | 2.4  | 1.4             | 93 |
| Rifampicin                | 22      | 3.0             | 1.6| 70    | 2   | 3.1     | 1.8             | 54 | 3    | 1.9 | 96  | 2    | 1.4  | 1.3             | 95 | 2    | 1.4 | 96  | 2    | 1.4  | 1.3             | 95 | 2    | 1.4 | 96  | 2    | 1.4  | 1.3             | 95 |
| Itraconazole and clarithromycin | 13  | 1.5             | 1.5| 75    | 6   | 0.6     | 1.3             | 100| 5    | 0.5 | 100 | 5    | 0.5  | 1.3             | 100| 5    | 0.5 | 100 | 5    | 0.5  | 1.3             | 100| 5    | 0.5 | 100 | 5    | 0.5  | 1.3             | 100|
| All                       | 62      | 4.9             | 1.9| 65    | 6   | 3.7     | 1.9             | 63 | 8    | 2.6 | 86  | 7    | 2.4  | 1.4             | 94 | 7    | 2.4 | 86  | 7    | 2.4  | 1.4             | 94 | 7    | 2.4 | 86  | 7    | 2.4  | 1.4             | 94 |

**SF<sub>active</sub>, active uptake scaling factor.**

*<sup>a</sup>n = 12* R-value evaluation of rifampicin DDI considered only cases with single-dose rifampicin, in which CYP3A4 induction is assumed negligible.

*<sup>b</sup>n = 4* R-value evaluation of clarithromycin DDI considered only.

Fig. 2. Observed versus predicted change in systemic exposure of the OATP1B1 substrate drugs when administered with (A) cyclosporine, (B) gemfibrozil, (C) rifampicin, and (D) itraconazole or clarithromycin. Data points represent AUCR (♦), R-value (♦), and the change in overall hepatic intrinsic clearance (−). Diagonal solid and dashed lines represent unity and 2-fold error, respectively. *n is number of combinations evaluated, and percentage represents number of predicted AUCRs within 2-fold of the observed AUCR.
using eq. 5) was underpredicted using the present in vitro transport and metabolic data, presumably due to discrepancy in the in vitro–in vivo extrapolation of transporter-mediated uptake activity (Watanabe et al., 2009; Jones et al., 2012; Menochet et al., 2012; Varma et al., 2012, 2013a; Jamei et al., 2014). Therefore, an empirical scaling factor for active uptake (SFactive) was applied to the extended clearance model (eq. 5) to recover the observed in vivo clearance. Although the individual scaling factors ranged from 1 (glyburide) to 101 (fluvastatin), the geometric mean SFactive (10.6) recovered clearance for 60 and 70% of victim drugs within 2- and 3-fold, respectively (Fig. 1B). With the three hepatocyte lots employed in this study, little interlot variability in active uptake was noted, and no clear trend in lot-specific activity was observed (Fig. 1), indicating that the in vitro underestimation of uptake clearance is consistent across hepatocyte lots. The potential reasons for the underestimation of the hepatic transporter activity may include downregulation of transporter protein and/or partial loss of functional activity in the in vitro system (Watanabe et al., 2009; Jones et al., 2012; Menochet et al., 2012; Varma et al., 2012, 2013a). Based on the protein quantification using liquid chromatography–tandem mass spectrometry, our laboratory showed higher expression of OATPs in the human cryopreserved liver tissue in comparison with 5-day culture of SCHH—with an estimated relative expression factor of about 1.7–2.5 (Kimoto et al., 2012). However, relative expression factor only partially explains the scaling factor noted in this study. A recent report showed significant loss in transporter expression and activity in human cryopreserved hepatocytes compared with fresh hepatocytes and may contribute to the in vitro–in vivo disconnect (Lundquist et al., 2014). Genetic polymorphism in SLCO1B1, particularly homozygous OATP1B1*15 variant, was reported to possess reduced transport activity (Nishizato et al., 2003; Niemi et al., 2011; Lai et al., 2012; Tomita et al., 2013), and the hepatocytes with such variant may underpredict in vivo clearance. Further investigation in these areas is warranted to understand the lower functional activity in the in vitro systems (Barton et al., 2013; Zamek-Gliszczynski et al., 2013; Li et al., 2014; Lundquist et al., 2014). Nevertheless, improved DDI predictions with mean and drug-specific SFactive (Fig. 3) corroborate the hypothesis of in vitro–in vivo discrepancy in transporter activity and justify the application of suggested correction factor (SFactive).

Multiple transporters and enzymes could be involved in the hepatic clearance for some of the victim drugs. For instance, rosvastatin hepatic uptake was suggested to be mediated by OATP1B1, OATP1B3, OATP2B1, and sodium-dependent taurocholate cotransporting polypeptide (Kitamura et al., 2008; Bi et al., 2013). Considerations to the relative contributions are expected to yield improved predictions and can be incorporated in the current model. In this study, we incorporated individual contribution of metabolic isozymes (CYP3A4, CYP2C8, and CYP2C9). However, due to the lack of definitive information for OATP isoforms for all the drugs, we assumed hepatic uptake is mediated only by OATP1B1. Generally, cyclosporine, gemfibrozil, and rifampicin show similar inhibition potency (IC50 or Ki values) for OATP1B1 and OATP1B3 (van Giersbergen et al., 2007; Gui et al., 2008;
Gertz et al., 2013; Prueksaritanont et al., 2014), and therefore, AUCRs are not expected to be largely impacted by varied individual isoform contribution.

Interestingly, predicted change in overall hepatic clearance due to P450 inhibition or induction was minimal compared with the change noted in gut extraction (Fig. 2, C and D). The differential impact of CYP3A4 inhibition on the gut extraction and overall hepatic clearance can be further exemplified with clinical observation of atorvastatin-itraconazole interactions—wherein intravenous itraconazole had no effect, whereas oral itraconazole coadministration increased atorvastatin AUC significantly (Mazza et al., 2000; Maeda et al., 2011). Presumably, hepatic uptake being the rate-determining step in the systemic clearance of several OATP substrates, change in only metabolic activity has a relatively smaller effect on the overall hepatic clearance, even for the drugs like atorvastatin, repaglinide, and glyburide that are completely metabolized (Maeda et al., 2011; Varma et al., 2013a, 2014). A systematic analysis suggested that inhibition of CYP3A4 or biliary clearance via MRP2 (pravastatin) and BCRP (rosuvastatin) alone by cyclosporine shows no notable effect on the overall hepatic clearance and thus the AUCRs. Intestinal metabolism contributes to the first-pass extraction of drugs, particularly CYP3A substrates, and therefore, interactions at the level of the intestine are believed to be significant following oral dose (Bjornsson et al., 2003). In the current study, minimal or no change in gut extraction was predicted for the interactions with cyclosporine and gemfibrozil due to their weak interaction potency against CYP3A4 (Fig. 2, A and B). However, the prediction accuracy of the AUCRs with CYP3A inducer (rifampicin) and inhibitor (itraconazole and clarithromycin) was significantly improved after considering $F_g/F_R$ ratio in addition to the change in overall hepatic clearance (Fig. 2, C and D). Collectively, the model predictions suggest that the net effect of increased CYP3A activity at the gut and decreased hepatic uptake mainly determine the magnitude of interactions with rifampicin, whereas inhibition of CYP3A activity in the intestine contributes significantly to interactions with itraconazole.

Cyclosporine was suggested to increase oral absorption of certain drugs by inhibiting intestinal efflux transporters (P-glycoprotein, BCRP, and MRP2), which may contribute to the observed AUCRs. However, due to complexity in the absorption kinetics, and lack of quantitative information on transporter expression and activity, change in $F_A$ was not captured in the current model. Underprediction of pravastatin and rosuvastatin (Biopharmaceutics Classification System (BCS)/Biopharmaceutics Drug Disposition Classification System (BDDCS) class 3 (Benet et al., 2011)) interactions with cyclosporine could be attributed to this (Varma et al., 2012; Jamei et al., 2014). Nevertheless, except for pravastatin and rosuvastatin, all victim drugs are highly permeable (as represented by class 1 and 2 of BCS/BDDCS), and efflux transporters have a minimal role in limiting the intestinal absorption, whereas solubility may limit complete absorption of certain drugs. Previous reports considered $F_A$/$F_R$ values of unity to predict inhibition DDIs (Yoshida et al., 2012). Although this conservative approach is useful in avoiding false-negative predictions, this may result in overprediction of AUCR. Appropriate assessments of change in $F_A$ may be derived based on PBPK models, which warrant further investigation and validation (Darwich et al., 2010; Fan et al., 2010).

For some of the victim drugs (e.g., statins), free liver concentrations are key determinants of efficacy due to localization of pharmacological targets in the hepatocytes. Pharmacokinetic-pharmacodynamic relationships are typically established assuming plasma concentration mirrors the intracellular concentration at target site, and therefore change in systemic pharmacokinetics is believed to translate to an equivalent effect on the target-site concentrations. However, OATP substrates are highly concentrated in the liver, and inhibition of hepatic uptake will have differential effects on plasma and liver concentrations. Although lack of clinical data would admittedly limit the validation, we predicted $K_{p,uu}$ in an attempt to assess the quantitative change in liver concentration in comparison with plasma concentration in several DDI situations. As noted in this work, except for DDIs associated with itraconazole and subtherapeutic doses of gemfibrozil (class A), all the other interactions (class B and C) result in a substantial drop in $K_{p,uu}$. These results have potential implications for clinical practice—particularly therapies using statins. Arguably, dose adjustments based on plasma exposure during comedication may avoid systemic adverse events such as myopathy and rhabdomyolysis, but could lead to lack of clinical efficacy due to reduced hepatic concentrations.

A strategy for model-based predictions of transporter and complex DDIs associated with transporter-enzyme interplay is proposed (Supplemental Fig. 2). A conservative assessment of hepatic transporter- or P450-mediated DDIs for new chemical entities can be achieved with static basic models incorporating in vitro and in vivo drug parameters. The R-value generally provides an oversimplification of the transporter-mediated DDI risk, assuming hepatic active uptake is responsible for 100% of systemic clearance (Giacomini et al., 2010; US FDA, 2012). Therefore, a predicted positive R-value would require further assessment using mechanistic static or dynamic models for quantitative DDI evaluation. This study demonstrates the applicability of the static mechanistic model in the prediction of complex DDIs associated with multiple enzyme- and transporter-mediated processes. As shown in this study, hepatic transport kinetics and enzymatic (P450) stability data of the victim drug obtained from in vitro systems such as SCHH and human liver microsomes can be used as inputs. However, due to the current knowledge gaps in the in vitro—in vivo extrapolation of uptake transporter kinetics, a scaling factor for active uptake may be needed to recover the in vivo intrinsic hepatic clearance—primarily assuming the metabolite/biliary clearance is accurately scalable. The scaling factor for active transport clearance can be derived based on intravenous or oral clinical pharmacokinetics data obtained early in the development (e.g., first-in-human study). However, when clinical pharmacokinetic data are not available, the in vitro transport and metabolism data along with the validated geometric mean $SP_{active}$ may be employed for prospective predictions of transporter-mediated and complex DDI situations. The drugs studied in this work are suggested in vivo probe drugs for testing clinical relevance of OATPs, CYP2C8, and CYP3A4 in the disposition of investigational drug (European Medicines Agency, 2012; US FDA, 2012). With the comprehensive nature of the proposed static model, we note that the AUCRs predicted in this work are similar to those obtained by the dynamic mechanistic modeling (Varma et al., 2012, 2013a,b, 2014; Jamei et al., 2014). The proposed
static model has the advantage of being simple and more transparent and can be valuable in quantitative predictions of DDI scenarios in the drug discovery and development.

In conclusion, where systemic clearance is determined by the hepatic uptake as well as metabolism or biliary secretion, mechanistic considerations assuming permeability-limited disposition and the simultaneous influence of all interaction mechanisms are needed to accurately predict transporter-mediated and complex DDIs. The proposed mechanistic model can be used for DDI risk assessment and to potentially avoid unnecessary clinical DDI studies. Finally, this study mechanistically explained majority of the clinically relevant DDIs of statins and other OATP substrates.

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Authorship Contributions

Participated in research design: Varma.
Conducted experiments: Bi, Lin, Kimoto.
Contributed new reagents or analytic tools: Varma.
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Wrote or contributed to the writing of the manuscript: Varma, Kimoto, Lin, Bi.

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