The aim of the present study was to establish a physiologically based pharmacokinetic (PBPK) model for coproporphyrin I (CP-I), a biomarker supporting the prediction of drug-drug interactions (DDIs) involving hepatic organic anion transporting polypeptide 1B (OATP1B), using clinical DDI data with an OATP1B inhibitor rifampicin (300 and 600 mg, orally). The in vivo inhibition constants of rifampicin used as initial input parameters for OATP1Bs ($K_{i,u,OATP1Bs}$) and multidrug resistance-associated protein two-mediated biliary excretion were estimated as 0.23 and 0.87 μM, respectively, from previous reports. Sensitivity analysis demonstrated that the $K_{i,u,OATP1Bs}$ and biosynthesis rate of CP-I affected the magnitude of the interaction. $K_{i,u,OATP1Bs}$ values optimized by nonlinear least-squares fitting were ~0.5-fold of the initial value. It was determined that the blood concentration-time profiles of four statins were well-predicted using corrected individual $K_{i,u,OATP1B}$ values (ratio of in vitro $K_{i,u(statin)}$ to in vitro $K_{i,u(CP-I)}$). In conclusion, PBPK modeling of CP-I supports dynamic prediction of OATP1B-mediated DDIs.

CPT Pharmacometrics Syst. Pharmacol. (2018) 7, 739–747; doi:10.1002/psp4.12348; published online on 30 September 2018.
yield relatively high false-positive rates. Therefore, various endogenous compounds have been proposed as surrogate probes to facilitate early clinical assessment of pharmacokinetic DDls, which could reduce the need for unnecessary DDI studies introducing established probe drugs.

Probe candidates for hepatic OATP1Bs have been reported, such as bilirubin and its glucuronides, bile acids, especially glucuronide and sulfate conjugates, glucuronide and sulfate conjugates of steroids, fatty acid dicarboxylates, and coproporphyrin I and III (CP-I and CP-III). Of these, CP-I and III, both the byproducts of heme synthesis, can support the evaluation of OATP1B-mediated DDls because of their specificity for OATP1B1 and OATP1B3, sensitivity, and the good association of the area under the plasma concentration-time curve (AUC) between CP-I and statins (pitavastatin, atorvastatin, and fluvastatin). Importantly, both plasma CP forms present negligible diurnal variation, which is a desirable characteristic of a biomarker.

Mechanistic physiologically based pharmacokinetic (PBPK) modeling has increasingly been used as a tool to predict DDls in recent years by combining the PBPK models of both victim and perpetrator. In this study, we aimed to establish a PBPK model for CP-I in which hepatic uptake and efflux processes are considered. Followed by hepatic uptake, CP-I undergoes biliary excretion via multidrug resistance-associated protein 2 (MRP2). Clinical data, obtained with the positron emission tomography probe [11C]-TIC-Me, has shown that MRP2-mediated biliary excretion can be significantly inhibited by RIF at a clinically relevant dose (600 mg). Such data suggest the DI potential of RIF against MRP2, but in vitro inhibition data can be problematic because of the high variability in the reported values of in vitro inhibition constants ($K_i$) for MRP2 (7.9–83 μM, as listed in the University of Washington Metabolism and Transport Drug Interaction Database). Overall, it was considered important to account for the involvement of both hepatic OATP1Bs and MRP2 in the interaction between CP-I and RIF. CP-I is also excreted into the urine, and its renal clearance is not significantly altered after the administration of RIF (600 mg).

We previously presented a stepwise protocol to analyze multiple clinical cases of DDI involving drug transporters using a standardized PBPK model structure and unified model parameters for each victim or perpetrator drug. Using this standardized protocol and a previously established model for RIF, we constructed a PBPK model for CP-I and optimized parameters, including $K_i$ for OATP1Bs, which could explain our clinical data showing a dose response with oral RIF at 300 and 600 mg. Subsequently, we evaluated the predictability of the DDls between statins and RIF in the same clinical dataset, taking into account the difference between the in vitro $K_i$ of RIF for statins and CP-I. Accounting for intersubstrate differences in $K_i$ is consistent with the recent report of Barnett et al., who considered the IC50 values of RIF for both CP-I and rosuvastatin in vitro and conducted mixed model analysis of clinical interaction data. Such considerations enhance the value of a PBPK model used to translate CP-I interaction data to other OATP1B substrates.

**METHODS**

**Overview of PBPK modeling and simulation strategy**

We previously reported a framework for constructing PBPK models. A PBPK model for CP-I was constructed by introducing a parameter for CP-I biosynthesis ($v_{syn}$) into our previously proposed PBPK model (Supplementary Text S1). The $v_{syn}$ was incorporated in the liver compartment; CP-I and CP-III are spontaneously generated from coproporphyrinogen I and III, which are synthesized from uroporphyrinogen I and III by uroporphyrinogen decarboxylase expressed in both liver and erythrocytes. In patients of porphyria cutanea tarda caused by a deficient hepatic activity of uroporphyrinogen decarboxylase due to hepatitis, the urinary ratio of CPs to uroporphyrins (spontaneously generated from uroporphyrinogens) was decreased to 0.28, whereas the ratio was 4.2 in healthy volunteers, suggesting > 90% of CPs in humans in normal condition is synthesized in the liver. Thus, we regarded the liver as the major organ for CP-I biosynthesis. The enterohepatic circulation (EHC) of CP-I was incorporated in the model according to a previous report of kinetic model analyses using the plasma concentration data and urinary and fecal excretion data of $^{14}$C-labeled CP-I and CP-III. Using the PBPK model and parameters for RIF reported by Asaumi et al., RIF-mediated inhibition of hepatic OATP1B and MRP2 was incorporated. Differential equations and other equation settings describing the PBPK model are presented in Supplementary Text S1. Finally, (i) simulation of blood concentration-time profiles of CP-I in the absence and presence of RIF, (ii) sensitivity analyses to examine the impact of change in each parameter on the simulation outcome, and (iii) parameter optimization by nonlinear least-squares fitting were performed.

Simulation designs for our clinical study (volunteers, drug administration, time course, and sampling) and model parameter settings are described in Supplementary Text S1. Details for uptake and efflux experiments using plated human hepatocytes (Figure S1) for the determination of hepatic membrane permeation parameters for CP-I are also described in Supplementary Text S1.

**Software**

The nonlinear least-squares fitting software, Napp version 2.31, was used in all the optimization and simulation processes applied in the present study. The weight for the nonlinear least-squares calculation was set as the square root of the original value. The weighted sum of squares (WSS) and the Akaike’s information criteria (AIC) shown in Eqs. 1 and 2, respectively, were used to evaluate the fitting results.

\[ WSS = \sum_{i=1}^{n} \frac{(y_i - y_i')^2}{y_i}, \]  
\[ AIC = n \times \ln WSS + 2m. \]

**Model predictability**

To quantitatively evaluate model predictability for blood concentration-time profiles, AUC ratio, and maximum blood concentration ($C_{max}$) ratio, commonly used accuracy
test criteria, the average fold errors (AFEs), were calculated, as shown in Eq. 3:

\[ AFE = 10^{\frac{\sum_{i=1}^{n} (\log_{10} \text{Observed}) - (\log_{10} \text{Predicted})}{n}} \]

where \( n \) is the number of observations.

RESULTS
Simulation of blood and liver concentration-time profiles of RIF using the reported PBPK model
As shown in Figure 1, the observed blood concentration-time profiles of RIF administered orally at 300 and 600 mg were reproduced by the simulation with the comprehensive PBPK model and parameters reported previously.19 The unbound concentration-time profile (the first hepatocyte compartment shown in dotted line represents a simulated blood unbound concentration-time profile. The broken line represents a simulated intrahepatic unbound concentration-time profile (the first hepatocyte compartment shown in Supplementary Text S1).

Setting of in vivo inhibition constants for OATP1Bs and MRP2 by RIF
To simulate the dynamic change in the blood concentration of CP-I caused by RIF, the in vivo \( K_{i,u,OATP1Bs} \) (0.23 \( \mu \)M)18 was set initially. Our in vitro experiments using OATP1B-expressing cells (Figure S2) suggested that the reported \( K_{i} \) for OATP1B1 (0.78 \( \mu \)M) and that for OATP1B3 (0.18 \( \mu \)M) using CP-I as a substrate tend to be lower than those using statins as substrates (1.1–2.8 \( \mu \)M for OATP1B1, 0.30–0.86 \( \mu \)M for OATP1B3; Table S2). Thus, we assumed some intersubstrate differences in the inhibitory characteristics by RIF, as reported previously.20 As part of the PBPK-enabled modeling exercise, we considered a range of in vivo \( K_{i,u,OATP1Bs} \) values (between 0.33-fold and 3-fold of the initial value) to support parameter sensitivity (Figure S3) and optimization by fitting

(Figure 2). In addition, the in vivo \( K_{i,u,MRP2} \) (0.87 \( \mu \)M), calculated based on the change in the biliary clearance of \([^{11}C]\)-TIC-Me,17 was used (see details in Supplementary Text S1).

Sensitivity analyses of model parameters to demonstrate the effects on the blood concentration-time profiles of CP-I
The blood concentration-time profile of CP-I following RIF administration was simulated (Figure S3) using the constructed PBPK model and parameters shown in Table 1 and Table S1. Sensitivity analyses of the hepatic overall intrinsic clearance, \( C_{\text{int,all}} \) (and correspondingly altered \( V_{\text{syn}} \)), according to the theoretical equation shown in Supplementary Text S1 showed that these parameters could change the turnover of CP-I and the magnitude of the interaction with RIF. As the \( C_{\text{int,all}} \) and \( V_{\text{syn}} \) are increased, the RIF-mediated interaction is increased regardless of the different preset \( \beta \) values of 0.8 (Figure S3a), 0.5 (Figure S3b), and 0.2 (Figure S3c). In addition, our sensitivity analyses also showed that the OATP1B inhibitor potency, reflected by \( K_{i,u,OATP1Bs} \) (Figure S3d–f), was a determinant of the interaction between RIF and CP-I.

To evaluate the effects of \( K_{i,u,MRP2} \) and other parameters relating to EHC (\( F_{g,Fg} \), \( k_{s} \), and \( k_{\text{trans}} \)), whose initial values were derived from the previous report,21 further sensitivity analyses were performed (Figure S3g–l). The \( K_{i,u,MRP2} \) slightly affected the CP-I blood concentration when the \( \beta \) value was set at 0.5 and 0.2 (Figure S3h,i). As the \( F_{g,Fg} \) decreased, RIF-mediated interaction is increased (Figure S3j–l). Little change in the magnitude of the interaction was observed in sensitivity analyses of \( k_{s} \) and \( k_{\text{trans}} \) (data not shown).

Previous evidences suggested that > 90% of CPs in humans in normal condition is synthesized in the liver.22 We performed sensitivity analyses setting the fraction of CP-I biosynthesis in the liver (\( f_{\text{syn}} \)) as 1, 0.9, and 0.8 (Figure S3m–o). Little change in the magnitude of the interaction was...
observed with these different \( f_{\text{syn}} \). Thus, we used \( f_{\text{syn}} = 1 \) in the following analyses.

Optimization of model parameters by fitting of the blood concentration-time profiles of CP-I
Because \( CL_{\text{int,all}} \), \( V_{\text{syn}} \), \( K_{\text{u,OATP1B}1} \), and \( F_{\text{g}} \) markedly affected the magnitude of interaction between CP-I and RIF (Figure S3a–f, j–l), these parameters were optimized by nonlinear least-square fitting to the CP-I blood concentration–time profiles in the absence and presence of RIF. This was done in vitro. The parameters \( V_{\text{syn}} \) and \( F_{\text{g}} \) were 35.1–43.1 l/h/kg, 0.212–0.442 nmol/h/kg, and 0.256–0.318, respectively, depending on the setting of \( \beta \) values (Table 2). The optimized values of \( K_{\text{u,OATP1B}1} \) ranged between 0.0824 and 0.106 \( \mu \)M, which were 0.36-fold to 0.46-fold of the initial value (0.23 \( \mu \)M).

Simulation of blood concentration-time profiles of CP-I using its optimized parameters and PBPK model incorporating the inhibition of OATP1B1s alone
To compare the contribution of OATP1B1s and MRP2 to the magnitude of the CP-I–RIF interaction, a simulation was performed using a PBPK model for CP-I, in which only the inhibition of hepatic OATP1B1s by RIF was incorporated. Using the parameters described in Table 2, with exclusion of \( K_{\text{u,MRP}2} \), the simulated CP-I blood concentrations (Figure S3a–c) were lower than those using the original PBPK model incorporating dual (OATP1B1s/MPR2) inhibition (Figure 2a–c). Such a model-dependent difference was more significant when the \( \beta \) value was set lower.

The observed and the simulated AUC 0–24 h and \( C_{\text{max}} \) of CP-I in the presence of RIF are summarized in Table 3. The simulated AUC and \( C_{\text{max}} \) using the PBPK model with dual OATP1B1s/MPR2-inhibition were around 96% and 102–108% of the observed values, respectively. In comparison, the simulated AUC and \( C_{\text{max}} \) using the PBPK model with OATP1B1 inhibition only was 75–85% and 79–89% vs. the observed values, respectively.

Prediction of the effect of RIF on blood concentration-time profiles of statins using their PBPK models and parameters including substrate-dependent \( K_{\text{u,OATP1B}1} \)
We evaluated the predictability of DDIs between four statins and RIF using our clinical data, as well as the \( K_{\text{u,OATP1B}1} \) for CP-I optimized by PBPK modeling (Table 2) and the ratio of \( K_{\text{i,u,OATP1B}1} \) for each statin to CP-I (Table S2). Previously reported PBPK models for pitavastatin, fluvastatin,}

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**Table 1** Parameters used in the simulation of the blood concentration–time profiles of CP-I in the absence and presence of RIF (Figure S3)

| ID | \( CL_{\text{int,all}} \) \( \mu \)l/h/kg | \( V_{\text{syn}} \) nmol/h/kg | \( R_{\text{diff}} \) | \( \gamma \) | \( f_{\text{ bile}} \) | \( k_{\text{transit}} \) h\(^{-1}\) | \( k_{\text{s}} \) h\(^{-1}\) | \( F_{\text{g}} \) | \( K_{\text{u,OATP1B}1} \) \( \mu \)M | \( K_{\text{u,MRP}2} \) \( \mu \)M |
|----|-----------------|-----------------|-------------|-----|----------|----------|----------|-------|-----------------|-----------------|
| A  | 33.3            | 0.153           | 0.8         | 0.035| 0.020    | 0.84     | 5.2      | 3.0   | 0.66            | 0.23            |
| B  | 33.3            | 0.125           | 0.5         | 0.035| 0.020    | 0.84     | 5.2      | 3.0   | 0.66            | 0.23            |
| C  | 33.3            | 0.105           | 0.2         | 0.035| 0.020    | 0.84     | 5.2      | 3.0   | 0.66            | 0.23            |

\( \beta = 0.8 \); \( \gamma = 0.8 \); \( \beta = 0.5 \); \( \gamma = 0.5 \); \( \beta = 0.2 \); \( \gamma = 0.2 \).
Table 2 Parameters fixed and optimized in the fitting of the blood concentration-time profiles of CP-I in the absence and presence of RIF (Figure 2)

| CL_int,all (l/h/kg) | ψ_syn | β | R_diff | γ | f_bile | k_transit | k_a | F_syn | F_syn | K_i,u,OATP1B1 | K_i,u,MRP2 |
|-------------------|--------|----|--------|----|--------|----------|-----|-------|-------|-------------|-------------|
| ID | Fixed | Fixed | Fixed | Fixed | Fixed | Fixed | Fixed | Fixed | Fixed | Fixed | Fixed |
| A | 35.1 ± 2.7 | 0.442 (0.345–0.567) | 0.8 | 0.035 | 0.020 | 0.84 | 5.2 | 3.0 | 0.256 ± 0.067 | 0.0824 ± 0.0105 | 0.87 | 1.73 | 19.1 |
| B | 38.3 ± 2.4 | 0.264 (0.219–0.315) | 0.5 | 0.035 | 0.020 | 0.84 | 5.2 | 3.0 | 0.309 ± 0.079 | 0.0965 ± 0.0123 | 0.87 | 1.75 | 19.5 |
| C | 43.1 ± 2.3 | 0.212 (0.182–0.245) | 0.2 | 0.035 | 0.020 | 0.84 | 5.2 | 3.0 | 0.318 ± 0.087 | 0.106 ± 0.013 | 0.87 | 1.81 | 20.2 |

β = CL_int,all/[PS_act,int] + PS_diff,int, R_diff = PS_diff,int/[PS_act,int, PS_diff,int], PS_diff,int = CL_int,met/(CL_int,bile + CL_int,all)  
AIC, Akaike information criterion; CP-I, coproporphyrin; RIF, rifampicin.  
*Calculated using the parameters including CL_int,all and F_syn according to Eqs. S7–S10. In the parentheses, the minimum and maximum values calculated using SDs of CL_int,all and F_syn are described.

Table 3 The blood AUC and C_max of CP-I estimated by PBPK modeling compared to the observation

| AUC_0–24h (nM h) | C_max (µM) |
|------------------|-----------|
| **Observation** | | |
| - | 23.9 ± 4.8 | 36.3 ± 6.7 | 1.61 ± 0.38 | 2.30 ± 0.35 |
| **Inhibition of OATP1Bs and MRP2** | | | | |
| - | 23.9 (95.8) | 34.8 (95.9) | 1.73 (100) | 2.36 (103) |
| **Inhibition of OATP1Bs (Figure S4)** | | | | |
| - | 20.3 (84.9) | 29.0 (79.9) | 1.43 (88.8) | 1.79 (77.8) |

In the parentheses, the estimation/observation ratios (%) are shown.

AUC, area under the plasma concentration-time curve; C_max, peak plasma concentration; CP-I, coproporphyrin I; MRP2, multidrug resistance-associated protein 2; OATP, organic anion transporting polypeptide; PBPK, physiologically based pharmacokinetic; RIF, rifampicin.

Data from the previous study.13

Rosuvastatin,26 and a newly constructed PBPK model for atorvastatin, whose structure is the same as pitavastatin and fluvastatin, leveraged their basic physiological and pharmacokinetic parameters (Table S1A,C). After obtaining some parameters by fitting to the control data alone (Table S1D), the blood concentration-time profile of each statin in the presence of RIF was simulated (Figure S3a–d) using substrate-dependent K_i,u,OATP1B1 shown in Table S1D, calculated by multiplying the K_i,u,OATP1B1 for CP-I by the ratio of in vitro K_i for OATP1B1 (statin/CP-I). AFEs for concentration-time predictions in the presence of 300 and 600 mg RIF were calculated to be 1.71/1.97, 1.87/2.42, 1.50/1.56, and 1.43/1.73 for pitavastatin, rosuvastatin, atorvastatin, and fluvastatin, respectively. The predicted AUC and C_MAX of statins were well-correlated with their observed AUC and C_MAX (Figure S3e,f).

On the other hand, when the prediction was performed using the in vitro K_i,u,OATP1B1 for CP-I (0.1 µM) without any correction (Figure S5a–d), AFEs were calculated to be 2.11/2.54, 1.92/2.53, 1.61/2.34, and 2.63/2.98 for pitavastatin, rosuvastatin, atorvastatin, and fluvastatin with 300 and 600 mg RIF, respectively. Therefore, the inhibitory effect of RIF was overestimated, except for rosuvastatin whose in vivo RIF K_i was not so different from CP-I. The predicted (without correcting K_i,u,OATP1B1) vs. observed AUC and C_MAX are shown in Figure S5e,f.

Finally, the AUC ratio and C_MAX ratio were calculated, as shown in Figure S5g–j. In this instance, with correction of the in vivo K_i based on differences of in vitro K_i, the AFE for predicted vs. observed AUC ratio was 1.20, and that for the C_MAX ratio was 1.36 (Figure S5g,h). Without correction of the in vivo K_i, based on differences of in vitro K_i, AFE values for the AUC ratio and C_MAX ratio were higher (1.74 and 1.39, respectively; Figure S5i,j).

**DISCUSSION**

In this study, a PBPK model for CP-I was constructed to provide mechanistic insight regarding the dose-dependent inhibition of OATP1B and MRP2 by RIF. Sensitivity analyses demonstrated that model parameters including the in vivo K_i,u,OATP1B1, ψ_syn, and CL_int,all, could affect CP-I blood concentration-time profiles following RIF. After optimization of these parameters by nonlinear least-squares fitting, blood concentration-time profiles of statins affected by RIF were well-predicted using substrate-dependent K_i values.

The initial value in our present analyses was based on the in vivo K_i,u,OATP1B1 values of RIF, which were estimated to be 0.23 µM (vs. pitavastatin) and 0.19 µM (vs. pravastatin) in our previous PBPK-DDI analyses.18 Parameter optimization yielded 0.37-fold to 0.48-fold of the initial K_i,u,OATP1B1 (0.082–0.11 µM in Table 2) to explain the CP-I blood concentration-time profiles with RIF (Figure 2). Some substrate-dependent differences in the sensitivity to OATP1B inhibitors in vitro, including RIF, have been reported.25 In order to support the intersubstrate difference suggested by the model-based...
PBPK Modeling of Coproporphyrin I
Yoshikado et al.

(a) Pitavastatin

Observed AUC (nM·h)
Predicted AUC (nM·h)

Observed C\text{max} (nM)
Predicted C\text{max} (nM)

Atorvastatin
Fluvastatin
Rosuvastatin
Pitavastatin

(b) Rosuvastatin

c) Atorvastatin

d) Fluvastatin

(e) Observed AUC (nM·h) vs. Predicted AUC (nM·h)
(f) Observed C\text{max} (nM) vs. Predicted C\text{max} (nM)

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In this study, we compared the inhibition profiles of RIF on blood concentration-time profiles of statins using their physiologically based pharmacokinetic (PBPK) models and parameters, including substrate-dependent $K_{i,u,OATP1B3}$. The blue (control), green (300 mg RIF, orally), and red (600 mg RIF, orally) circles represent the observed blood concentrations of statins (mean ± SD, $n = 8$). The blue solid lines (control) represent fitted blood concentration-time profiles of statins after optimization of parameters in their PBPK models. The green and red broken lines (+RIF) represent predicted blood concentration-time profiles of statins. Pitavastatin, rosvastatin, atorvastatin, and fluvastatin. The used parameters, including substrate-dependent $K_{i,u,OATP1B3}$ values are shown in Table S1A,C,D. Comparison of the predicted and observed area under the plasma concentration-time curve (AUC) and peak plasma concentration ($C_{max}$) of statins in the presence and absence of RIF. The AUC and $C_{max}$ values of coproporphyrin (CP-I) are also shown. Dashed and dotted lines denote unity and threefold boundaries, respectively.

By comparing the simulation results using the model incorporating dual OATP1B/MRP2 inhibition (Figure 2), to that incorporating OATP1B inhibition only (Figure S4), 75–85% of the observed AUC and 78–89% of the observed $C_{max}$ were explained by inhibition of OATP1B1 (Table 3), and the remaining by inhibition of MRP2. Thus, OATP1B1 had a major contribution to the magnitude of the CP-I-RIF interaction, however, the magnitude suggests that the influence of MRP2 inhibition on CP-I pharmacokinetics should not be ignored during the assessment of DDIs.

Using the first model with an initial parameter setting (Figure S3), sensitivity analysis of $K_{i,u,MRP2}$ from 0.33 to 3-fold of the initial value affected blood concentrations in the presence of RIF (especially when $\beta = 0.8$) less significantly than $K_{i,u,OATP1B3}$. Actually, the contribution of RIF-mediated inhibition of MRP2 was estimated to be ~10% of the AUC increase at most ($\beta = 0.5$ and 0.2). The reduced impact of $K_{i,u,MRP2}$ on CP-I blood concentrations is expected because of (i) increased sinusoidal efflux of CP-I is offset by (ii) the decreased EHC (with $F_{g,F_{g}}$ of 0.66) of CP-I, both due to the inhibition of MRP2-mediated biliary excretion. On the other hand, the effect of MRP2-inhibition was more clearly observed when using the final model with an optimized parameter setting (Figure 2), because (i) a larger $\nu_{syn}$ setting led to the more significant effect of RIF, and (ii) a smaller $F_{g,F_{g}}$ setting (0.26–0.32) led to less contribution of EHC.

For some time it has been known that the urinary CP-I/CP-III ratio is associated with ABC2 carriers and it has been suggested that the ratio can serve as a MRP2 trait measure. The increase in CP-I/CP-III ratio associated with Dubin–Johnson syndrome might result from increased plasma concentration and urinary excretion of CP-I due to the diminished MRP2-mediated biliary excretion of CP-I. Although MRP2 is also expressed on the human kidney proximal tubule epithelia, inhibition of renal MRP2 was not incorporated in our PBPK model because the renal clearance of CP-I is not affected by RIF.

Yoshida et al. summarized the effects of Rotor Syndrome (RS) on the urinary excretion of CP-I from six previous articles. It was concluded that the ratio of urinary excretion in healthy control (non-RS) subjects vs. patients with RS (non-RS/RS) was comparable across the various studies.
for CP-I (0.18 and 0.78 μM in Table S2; 0.25 and 3.3 μM from the previous reports\textsuperscript{32,33}) and those for statins as other OATP1B substrates (0.30–2.8 μM in Table S2; 0.30–5.7 μM in the Drug Interaction Database). In vivo $K_{i,u}$ values estimated using the empirical models should not be applied to other probes directly, because (i) the hepatic clearance used in the empirical models is a hybrid parameter containing the hepatic blood flow and intrinsic clearances (uptake, basolateral efflux, metabolism, and biliary excretion) for CP-I. By not considering the site of inhibition by RIF, one could greatly impact the estimation of in vivo $K_{i,u}$; and (ii) the former studies did not consider the site of biosynthesis for CP-I. Actually, the liver is the major organ responsible for the biosynthesis of CP-I (see METHODS). Therefore, hepatic MRP2 function as well as OATP1Bs should be important determinants for CP-I blood concentration. This should also affect the estimation of in vivo $K_{i,u}$.

Thus, our PBPK modeling approach for CP-I has the advantage and advancement from the previous empirical modeling approach in terms of consideration of the site of biosynthesis, rate-determining process in the hepatic elimination, and the inhibition mechanisms, which enables the prediction of complex DI involving a new chemical entity (NCE) via hepatic transport processes (OATP1Bs and MRP2) based on in vitro information, including substrate-dependent $K_{i}$, as we propose a workflow in Figure 4. (I) Concentration-time profiles of given NCE obtained in an exploratory, first-in-human study (phase-0) or in a phase-1 study (dose escalation study) would provide an opportunity to construct a PBPK model, in which several parameters determined in vitro and in silico are incorporated according to our previous standardized method.\textsuperscript{18} Using the NCE’s PBPK model and our PBPK model for CP-I, in vivo $K_{i,OATP1B}$ for CP-I can be obtained by analyzing its concentration-time profiles in the absence and presence of the NCE. (II) In vitro $K_{i,OATP1B}$ values for CP-I and for probe substrate drugs (e.g., statins) would be needed to calculate in vivo $K_{i,OATP1B}$ for the drugs according to the equation shown in Figure 4. (III) Finally, using the calculated in vivo $K_{i,OATP1B}$ for the probe substrate drugs, PBPK modeling and simulation would support the prediction of changes in substrate concentration-time profiles, AUC, and $C_{\text{max}}$ caused by the NCE. Although some problems ascribed to experimental techniques in vitro remain to be solved (i.e., difficulty in obtaining reliable in vitro $K_{i,u}$ of highly lipophilic compounds due to nonspecific binding), translation of the effect of an NCE on CP-I pharmacokinetics to effects on clinically used drugs is gradually becoming more accepted.\textsuperscript{5,7,12,20,34}

In conclusion, the PBPK modeling approach presented herein provides a deeper understanding of the mechanisms governing CP-I pharmacokinetics and enables complex analyses of the dose-dependent inhibitory effects of RIF on the hepatic OATP1Bs/MRP2-mediated transport of CP-I. Based on the in vivo $K_{i}$ for CP-I optimized by PBPK modeling, and the ratio of in vitro $K_{i}$ for each statin to CP-I, we showed that it is possible to accurately predict the blood concentration-time profiles of OATP1B probe drugs affected by RIF at two dose levels. Presently, the vision is to expand the use the model to include additional OATP1Bs inhibitors beyond RIF.

**Figure 4.** Scheme of the workflow for predicting drug-drug interactions using coproporphyrin (CP)-I as an endogenous biomarker. (I) Concentration-time profiles of a new chemical entity (NCE) obtained in an exploratory, first-in-human study (phase-0) or in a phase-1 study (dose escalation study) would provide an opportunity to construct a PBPK model, in which several parameters determined in vitro and in silico are incorporated according to our previous standardized method.\textsuperscript{18} Using the NCE’s PBPK model and our PBPK model for CP-I, in vivo $K_{i,OATP1B}$ for CP-I can be obtained by analyzing its concentration-time profiles in the absence and presence of the NCE. (II) In vitro $K_{i,OATP1B}$ values for CP-I and for probe substrate drugs (e.g., statins) would be needed to calculate in vivo $K_{i,OATP1B}$ for the drugs according to the equation shown in Figure 4. (III) Using the calculated in vivo $K_{i,OATP1B}$ for the probe substrate drugs, PBPK modeling and simulation will enable us to predict changes in their concentration-time profiles, area under the plasma concentration-time curve (AUC) and peak plasma concentration ($C_{\text{max}}$) caused by the NCE.

**Supporting Information.** Supplementary information accompanies this paper on the CPT: Pharmacometrics & Systems Pharmacology website. (www.psp-journal.com).

**Figure S1.** Uptake and efflux profiles of CP-I in plated human hepatocytes.

**Figure S2.** Saturation of CP-I uptake by OATP1B1, and inhibitory effects of RIF on OATP1B1-mediated and OATP1B3-mediated uptakes of CP-I and statins in vitro.

**Figure S3.** Sensitivity analyses of model parameters to examine effects on the blood concentration-time profiles of CP-I in the absence and presence of RIF using the PBPK model incorporating the inhibitions of OATP1Bs and MRP2.

**Figure S4.** Simulation of blood concentration–time profiles of CP-I in the absence and presence of RIF using parameters optimized as shown in Figure 2 and the PBPK model incorporating the inhibition of OATP1Bs alone.

**Figure S5.** Prediction of the effect of RIF on blood concentration–time profiles of statins using their PBPK models and parameters including substrate-dependent $K_{i,OATP1B}$ (without correction of the in vivo $K_{i}$ based on differences of in vitro $K_{i}$).

**Table S1.** Parameters used in the PBPK model for CP-I and statins.
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