A Study on Pharmacokinetics of Bosentan with Systems Modeling, Part 1: Translating Systemic Plasma Concentration to Liver Exposure in Healthy Subjects^S

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ABSTRACT

Understanding liver exposure of hepatic transporter substrates in clinical studies is often critical, as it typically governs pharmacodynamics, drug-drug interactions, and toxicity for certain drugs. However, this is a challenging task since there is currently no easy method to directly measure drug concentration in the human liver. Using bosentan as an example, we demonstrate a new approach to estimate liver exposure based on observed systemic pharmacokinetics from clinical studies using physiologically based pharmacokinetic modeling. The prediction was verified to be both accurate and precise using sensitivity analysis. For bosentan, the predicted

pseudo steady-state unbound liver-to-unbound systemic plasma concentration ratio was 34.9 (95% confidence interval: 4.2, 50). Drug-drug interaction (i.e., CYP3A and CYP2B6 induction) and inhibition of hepatic transporters (i.e., bile salt export pump, multidrug resistance-associated proteins, and sodium-taurocholate cotransporting polypeptide) were predicted based on the estimated unbound liver tissue or plasma concentrations. With further validation and refinement, we conclude that this approach may serve to predict human liver exposure and complement other methods involving tissue biopsy and imaging.

Introduction

The pharmacokinetics (PK) of many drugs can be influenced by transporters. Since transporter-mediated disposition [e.g., organic anion-transporting polypeptides (OATPs)] can be independent of the substrate concentration gradient, drugs may accumulate or be excluded from tissues. Therefore, even without considering factors like membrane potential, it may not be accurate to assume that unbound tissue concentration is equivalent to unbound plasma concentration ($K_{pu} = 1$). Since both transporters and metabolism affect drug concentration in the liver, there are two challenges for drug discovery: 1) predicting systemic exposure, which is dependent on the interplay between transporters and metabolism in the liver, and 2) predicting liver exposure, which may drive pharmacodynamics, toxicity, and drug-drug interaction (DDI). In the drug development phase, even with clinical data, predicting liver exposure may still be ambiguous in relating observed plasma PK to tissue exposure-driven clinical outcomes.

To address the challenge of understanding human liver exposure, positron emission tomography (PET) studies for transporter substrates have been developed (Shimizu et al., 2012; Gormsen et al., 2016). In addition to excessive cost and potential difficulties in labeling compounds,

the usefulness of this approach is still limited to compounds with minimal metabolism. For the majority of drugs with significant metabolism, the PET data are confounded by metabolite signals. As such, in the foreseeable future, translating observed systemic concentration to liver concentration with mechanistic modeling [e.g., physiologically based pharmacokinetic (PBPK) models] may be one of the most effective tools to enable a greater understanding of human liver exposure. However, this approach has its limitations. For example, PBPK models may become unidentifiable with non-ideal systemic data. In such cases, many sets of parameter values can equally well describe the systemic plasma data, but lead to different liver predictions (Li et al., 2016).

From such a perspective, bosentan is a great example because its systemic exposure data can provide enough information for PBPK modeling to confidently predict liver concentrations. Bosentan is a dual endothelin receptor (ET) antagonist used to treat pulmonary arterial hypertension (Dingemans and van Giersbergen, 2004). Bosentan is an Extended Clearance Classification System 1B compound (El-Kattan et al., 2016). In humans, bosentan is transported into the liver by uptake transporters including OATP1B1 and OATP1B3 (Treiber et al., 2007) and is then metabolized by CYP3A and CYP2C9 (Dingemans and van Giersbergen, 2004), with minimal unchanged drug recovered in urine and feces after intravenous dosing (Weber et al., 1999b). Although the PK profile of bosentan is more complicated than other compounds (e.g., nonlinear disposition and distribution; Weber et al., 1996), this can be

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ABBREVIATIONS: BSEP, bile salt export pump; CI, confidence interval; DDI, drug-drug interaction; DILI, drug-induced liver injury; ET, endothelin receptor; MCMC, Markov chain Monte Carlo; MRP, multidrug resistance-associated protein; NTCP, sodium-taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic; PET, positron emission tomography; PK, pharmacokinetics; RBC, red blood cell.

TABLE 1
Bosentan clinical data included in the PBPK modeling exercise

Dosed Compound(s)	Dosing Route	Dosing Amount	Reference
		mg	
Bosentan	Intravenous infusion (5 min)	10, 50, 250, 500, or 750	Weber et al. (1996)
Bosentan	Intravenous infusion (15 min)	250	Weber et al. (1999b)
Bosentan	Tablet after high-fat meal	500	U.S. Food and Drug Administration (2001)
Bosentan	Tablet after high-fat meal	125	Dingemanse et al. (2002)
Bosentan	Tablet	500	Weber et al. (1999c)
Bosentan	Tablet	62.5	van Giersbergen et al. (2002b)
Bosentan (twice daily) and tadalafil (once daily)	Tablet	Bosentan: 125; tadalafil: 40	Wrishko et al. (2008)
Bosentan (twice daily) and warfarin (once on day 6)	Tablet	Bosentan: 500; racemic mixture of warfarin: 26	Weber et al. (1999a)

addressed with a carefully calibrated model. Bosentan is not characterized by significant biliary excretion or enterohepatic recirculation, which would have made data interpolation very challenging.

Understanding bosentan liver exposure is also important for predicting cytochrome P450 (P450) induction and inhibition. Several groups have reported that bosentan is an inducer for P450s (van Giersbergen et al., 2002a; Dingemanse and van Giersbergen, 2004; Fahmi et al., 2008; Srinivas, 2016; Sun et al., 2017). Results of in vitro studies also show that bosentan inhibits the bile salt export pump (BSEP), multidrug resistance-associated proteins (MRPs) 3 and 4 (Morgan et al., 2013), and sodium-taurocholate cotransporting polypeptide (NTCP) (Leslie et al., 2007), which may result in drug-induced liver injury (DILI) (Leslie et al., 2007; Morgan et al., 2013). Hepatic P450 induction and transporter inhibition is likely driven by liver concentration, so a highly confident prediction of liver concentration is critical to understand clinically observed DDI and DILI.

In this study, we developed a PBPK model for bosentan incorporating its various PK properties and we generated liver exposure and its confidence intervals (CIs) using a Markov chain Monte Carlo (MCMC) approach. The key parameters were determined either in preclinical assays or by simultaneously fitting data from eight independent clinical studies (Table 1) to avoid potential model misspecifications due to improper assumptions.

Materials and Methods

A Mechanistic Model to Analyze PK Data

Framework. A new PBPK model (scheme in Fig. 1) was developed based on a published structure (Li et al., 2014). Table 2 provides all of the parameters with fixed values, except for the physiologic parameters listed in Supplemental Table 1. Equations and all other modeling details not covered in the text are presented in the Supplemental Material.

Systemic Circulation and Nonliver Tissue Distribution. The arterial blood, venous blood, and lung are modeled as systemic blood, which is then split into systemic plasma and red blood cells (RBCs). Due to potential nonlinear binding kinetics, instead of assuming the constant plasma unbound fraction ($f_{u,p}$) or blood-to-plasma ratio ($R_{B/P}$), we use the kinetic model to describe binding in plasma and RBCs. For example, binding in the plasma was modeled with mass balances of unbound concentration, available binding site concentration, and bound concentration (eqs. 1–3, respectively):

$$\frac{dC_{\text{unbound}}}{dt} = -k_{\text{on}} \cdot C_{\text{unbound}} \cdot C_{\text{available-site}} + k_{\text{off}} \cdot C_{\text{bound}} \quad (1)$$

$$\frac{dC_{\text{available-site}}}{dt} = -k_{\text{on}} \cdot C_{\text{unbound}} \cdot C_{\text{available-site}} + k_{\text{off}} \cdot C_{\text{bound}} \quad (2)$$

$$\frac{dC_{\text{bound}}}{dt} = k_{\text{on}} \cdot C_{\text{unbound}} \cdot C_{\text{available-site}} - k_{\text{off}} \cdot C_{\text{bound}} \quad (3)$$

The binding in RBCs was modeled similarly; passive permeation ($CL_{\text{systemic,blood,pass}}$) was assumed between RBCs and plasma. All components in the systemic

blood are connected with their counterparts in the liver and small intestine villi blood.

k_{on} rates for all binding processes in this study were fixed at 10^9 1/M/s (3600 1/nM/h) assuming the diffusion limited reaction (Alberty and Hammes, 1958). k_{off} and the total concentrations of the binding site in plasma and RBCs were estimated by fitting the in vitro unbound fraction and blood-to-plasma ratio at various concentrations (Supplemental Fig. 1) with a mechanistic model. Because the model is not sensitive to $CL_{\text{systemic,blood,pass}}$, this parameter was fixed at the product of total systemic RBC surface area and a permeability approximated using hepatocytes; details are provided in the Supplemental Material.

For nonliver tissues, instantaneous equilibrium between tissue and unbound systemic plasma was assumed, defined by in silico predicted $K_{p,i}$ (i.e., total tissue-to-unbound plasma ratio) values (Rodgers and Rowland, 2006). Target-mediated drug disposition has been proposed in previous studies (Mager and Jusko, 2001; Volz et al., 2017), but it is unlikely that the targets (i.e., ETs) or their internalization will eliminate the compound. As such, target-mediated drug disposition was modeled as a specific binding process to the ET in the plasma compartment, with parameters optimized by fitting clinical data.

The Liver. The model includes five sequential liver segments, each containing three components: plasma, RBCs, and tissue. Each component is further divided into three subcomponents to represent the unbound compound, bound compound, and available binding site. There are hepatic active uptake, active basal efflux, and passive diffusion between plasma and tissue, and metabolism within the tissue. The biliary excretion was assumed to be minimal for bosentan based on the fact that 1) in vitro sandwich cultured human hepatocytes showed no biliary excretion (data provided in part 2 of this study; Li et al., 2018), and 2) minimal compound is excreted into feces after intravenous dosing in humans (Weber et al., 1999b). With the exception of passive diffusion clearance ($CL_{\text{liver,pass}}$), hepatic processes were assumed to follow Michaelis–Menten kinetics. Among Michaelis–Menten constants, $K_{M,\text{liver,uptake}}$ and $K_{M,\text{liver,metabolism}}$ were fixed at values based on in vitro assays. Due to low confidence in the in vitro values, $K_{M,\text{liver,efflux}}$ was estimated by fitting clinical data, together with maximal reaction rates ($k_{\text{liver,uptake}}$, $k_{\text{liver,efflux}}$, $k_{\text{liver,metabolism}}$), and $CL_{\text{liver,pass}}$. Blood binding parameters share the same values as those in circulating blood. Intracellular binding parameters are fixed at values estimated from in vitro hepatocyte assays in part 2 of this work (Li et al., 2018).

Absorption Parameters. Oral absorption was modeled using a semi-mechanistic model with a first-order rate constant (k_a) and fraction absorbed (F_a). An enterocyte compartment is created between the dissolved drug compartment and small intestine villi blood. We assume there are passive diffusion ($CL_{\text{enterocyte,pass}}$) and active efflux ($CL_{\text{enterocyte,efflux}}$) between enterocytes and small intestine villi blood, and metabolism in the enterocytes. Binding in villi blood was modeled the same as that within systemic and liver blood. The fraction absorbed (F_a) was determined using clinical ^{14}C data (with details provided in the Supplemental Material). Two different k_a values under fasted and fed conditions, $CL_{\text{enterocyte,pass}}$, $CL_{\text{enterocyte,efflux}}$, and enterocyte intracellular free fraction ($f_{u,\text{enterocyte}}$) were estimated by fitting clinical data. Although apparent K_M values for enterocyte and liver metabolism may be different due to potentially different P450s involved in the tissues, we assumed that $K_{M,\text{enterocyte,metabolism}}$ shared the same value with $K_{M,\text{liver,metabolism}}$ based on the fact that the in vitro CYP3A and CYP2C9 had similar K_M values (Shen et al., 2009), whereas the metabolic rate was scaled from $k_{\text{liver,metabolism}}$ based on P450 abundances in the human liver and gut (details provided in the Supplemental Material).

Fig. 1. Schematic diagram of a PBPK model for bosentan. Only two liver segments are presented in this scheme, but there are five segments in the model. Nonliver tissue type II represents tissues whose venous blood enters the portal vein, whereas type I represents the remaining nonliver tissues.

Induction Parameters. In vivo P450 induction is described using a turnover model. Because bosentan induces P450s via pregnane X receptor agonism (van Giersbergen et al., 2002a), we assumed that different P450s involved in hepatic and intestinal metabolism share the same induction $E_{\max}$

and EC_{50} values, which is supported by the similar values identified from the in vitro CYP3A4 and CYP2B6 activity assay described below. $E_{\max}$ was estimated by fitting clinical data of bosentan and victim drugs (see below), whereas EC_{50} was fixed at an average value from the in vitro assay

TABLE 2
Parameters with fixed values in the described bosentan PBPK model

Parameter	Value	Source	Parameter	Value	Source
pKa	5.2 (acidic)	In house	k_{on} (1/nM/h)	3600	Alberty and Hammes (1958)
logD _{7.4}	1.3	In house	$k_{off,plasma}$ (h ⁻¹)	8.18×10^7	Supplemental Material
MW (g/mol)	551.6	In house	$k_{off,RBC}$ (h ⁻¹)	1.25×10^7	Supplemental Material
$K_{M,liver,uptake}$ (nM)	4343	SCHH	$k_{off,liver,tissue}$ (h ⁻¹)	4.80×10^7	SCHH
$K_{M,liver,metabolism}$ (nM)	6.9×10^4	Shen et al. (2009)	Binding site in plasma (nM)	1.14×10^6	Supplemental Material
$K_{M,enterocyte,metabolism}$ (nM)	6.9×10^4	Shen et al. (2009)	Binding site in RBCs (nM)	4.11×10^4	Supplemental Material
Ratio of $k_{enterocyte,metabolism}$ to $k_{liver,metabolism}$	0.0260	Supplemental Material	Binding site liver tissue (nM)	3.67×10^5	SCHH
F_a , 62.5-mg tablet	0.973	Supplemental Material	CL _{systemic,blood,pass} (l/h)	1.12×10^3	Supplemental Material
F_a , 125-mg tablet	1	Supplemental Material	CL _{liver,blood,pass} (l/h)	72.5	Supplemental Material
F_a , 500-mg tablet	0.823	Supplemental Material	CL _{villi,blood,pass} (l/h)	2.43	Supplemental Material
F_a , 125 mg high fat	1	Supplemental Material	P450 $k_{enterocyte,degradation}$ (h ⁻¹)	0.0300	Yang et al. (2008)
F_a , 500 mg high fat	1	Supplemental Material	P450 $k_{liver,degradation}$ (h ⁻¹)	0.0250	Quinney et al. (2010)
Human HCT	0.519	In house	Induction EC ₅₀ (nM)	1000	Hepatocyte induction assay
IC _{50,NTCP} (nM)	2.4×10^4	Leslie et al. (2007)	IC _{50,MRP3} (nM)	4.2×10^4	Morgan et al. (2013)
IC _{50,BSEP} (nM)	2.3×10^4	Morgan et al. (2013)	IC _{50,MRP4} (nM)	2.2×10^4	Morgan et al. (2013)

HCT, hematocrit; SCHH, sandwich cultured human hepatocyte.

Fig. 2. (A-F) Total systemic plasma concentrations of bosentan after intravenous dosing. The solid lines and shaded areas represent the median and 95% CI of the simulations generated with parameter values identified in Markov chain Monte Carlo. The circles represent mean data from clinical studies.

Fig. 3. (A-H) Total systemic plasma concentrations of bosentan after oral dosing. (A-F) Subplots show the multiple oral dosing with different amounts. (G and H) Subplots show the single oral dosing with a high-fat meal. The solid lines and shaded areas represent the median and 95% CI of the simulations generate with parameter values identified in Markov chain Monte Carlo. The circles represent mean data from clinical studies. BID, twice daily; QD, once daily.

(i.e., 1000 nM). The P450 degradation rate ($k_{\text{degradation}}$) was calculated as $\ln(2)$ divided by the half-life. Because there are currently no published clinical data regarding CYP2C9 half-life, it is assumed that the degradation rate ($k_{\text{liver, degradation}}$) of CYP2C9 equals that of CYP3A4, estimated from a

clinical CYP3A4 inactivation study (27.7 hours; Quinney et al., 2010). The enterocyte half-life (23.1 hours; Yang et al., 2008) was applied to enterocyte P450s, assuming that half-life values of P450s are determined by the shorter half-life of enterocytes.

Fig. 4. (A–H) Total systemic plasma concentrations of tadalafil on day 1 (A and B) and day 10 (C and D), *S*-warfarin (E and F), and *R*-warfarin (G and H). Subplots (A), (C), (E), and (G) represent PK in the absence of bosentan, whereas subplots (B), (D), (F), and (H) represent PK in the presence of bosentan. The solid lines and shaded areas represent the median and 95% CI of the simulations generate with parameter values identified in Markov chain Monte Carlo. The circles represent mean data from clinical studies. BID, twice daily; QD, once daily.

BSEP, MRP, and NTCP Inhibition. Competitive inhibition of four transporters was calculated independently based on simulated bosentan unbound plasma (for NTCP) or intracellular (for BSEP and MRP) concentrations and in vitro IC_{50} values (Leslie et al., 2007; Morgan et al., 2013). Maximal inhibition

was assumed to be 100%. To our knowledge, there is currently no supporting evidence that inhibition affects bosentan exposure.

Victim Drugs. A reduced PBPK model was developed for victim drugs (i.e., tadalafil and warfarin) codosed with bosentan (Supplemental Material). Published

TABLE 3

Median values and 95% CIs of optimized parameters in the bosentan PBPK model
Values and CIs were estimated by fitting clinical data.

Parameter	Median (95% CI)
ET k_{off} (h^{-1})	1.61×10^4 (7200, 3.4×10^4)
ET total concentration (nM)	2750 (1400, 4900)
$\text{CL}_{\text{liver,pass}}$ (l/h)	23.9 (13, 44)
$k_{\text{liver,uptake}}$ (nmol/h)	4.78×10^6 (3.8×10^6 , 6.5×10^6)
$k_{\text{liver,metabolism}}$ (nmol/h)	1.00×10^6 (8.0×10^5 , 1.3×10^6)
$k_{\text{liver,efflux}}$ (nmol/h)	6.00×10^4
$K_{\text{M,liver,efflux}}$ (nM)	1.48×10^6
k_a (h^{-1})	0.932 (0.81, 1.3)
k_a (meal) (h^{-1})	0.759 (0.64, 1.1)
$f_{\text{u,enterocyte}}$	0.964 (0.50, 1.0)
$\text{CL}_{\text{enterocyte,pass}}$ (l/h)	2.31×10^{-5} ($<1.1 \times 10^{-4}$)
$\text{CL}_{\text{enterocyte,efflux}}$ (l/h)	6.80×10^{-5} ($<1.1 \times 10^{-4}$)
Clinical induction E_{max}	1.36 (1.0, 1.7)
Tadalafil $F_a F_g$ with bosentan	0.868 (0.70, 1.0)
S-warfarin $F_a F_g$ with bosentan	1.10 (0.99, 1.2)
R-warfarin $F_a F_g$ with bosentan	1.00 (0.83, 1.3)

Medians and CIs are not provided for the parameters with high uncertainty (i.e., the range of the approximated CI is greater than 20 magnitude). The globally optimized values (and upper bound if possible) are provided instead.

bosentan induction DDI studies with other drugs were not included in the modeling, since it is challenging to simulate victim liver exposure (e.g., transporter substrates: simvastatin and glyburide) and because victim drugs also affect bosentan exposure (e.g., glyburide reduces bosentan exposure, and sildenafil increases bosentan exposure). For victim drugs, bosentan may change its gut metabolism; hence, we assumed that their F_g (i.e., the fraction escaped from gut metabolism) values are different with and without bosentan. Since it is difficult to separate F_a from F_g , $F_a F_g$ was modeled as a single parameter. $F_a F_g$ was fixed at 1 in the absence of bosentan but was fitted against clinical data in the presence of bosentan. Parameters for victim drugs in the absence of bosentan are listed in Supplemental Table 2.

Parameter Optimization and Prediction of Liver Exposure

Bosentan data from eight clinical studies were included for parameter optimization (Table 1). The model was implemented in MATLAB 2016a software (MathWorks, Natick, MA). Parameter estimation was performed with differential evolution, while the uncertainty was quantified using an MCMC approach. MCMC provides ranges of parameter values that are able to reasonably describe the data. We randomly sampled 1000 sets of parameter values from all values (8×10^5 sets) identified in MCMC that adequately describe systemic plasma data. One-thousand simulations using sampled parameter values were generated, such that uncertainty in parameter estimation was reflected in the simulations.

In Vitro Induction Assay and Modeling

An in vitro hepatocyte induction study was performed to understand whether the P450 induction could be accurately predicted using primary hepatocytes. The data were analyzed using a mechanistic model that combines the sandwich cultured human hepatocyte model (Li et al., 2018) and the P450 turnover model mentioned above. Details are provided in the Supplemental Material.

Results

Fitting Clinical Systemic Data and Estimating Parameters

With optimized parameter values, the model can reasonably describe the mean systemic exposures of both bosentan and victim drugs after intravenous or oral administration with various doses (Figs. 2–4) obtained from several studies. Parameters can be confidently estimated (Table 3), with the exception of $\text{CL}_{\text{enterocyte,pass}}$, $\text{CL}_{\text{enterocyte,efflux}}$, $k_{\text{liver,efflux}}$, and $K_{\text{M,liver,efflux}}$. This is potentially due to correlation among the different parameters or to the insensitivity of simulations to these parameters.

Simulating Liver Exposure

Despite the fact that some parameters cannot be confidently identified by fitting systemic data, the predicted liver exposure is still reasonably precise (Fig. 5). The predicted pseudo steady-state ratio between unbound liver tissue and unbound systemic plasma concentrations ($K_{\text{pu,liver}}$; i.e., the ratio during the elimination phase of systemic PK) after 125 mg bosentan twice-daily dosing was 34.9 (95% CI, 4.2, 50). The time course of unbound liver tissue to unbound systemic plasma after 62.5, 125, or 500 mg twice-daily dosing is provided in Fig. 5, B, E, and H, in which the median values change between 20 and 40. Please note that the $K_{\text{pu,liver}}$ calculated here is the ratio of unbound liver tissue to unbound systemic plasma concentration, but not to unbound liver plasma concentration. Hypothetically, there is a difference in concentration between systemic plasma and liver plasma due to liver extraction.

P450 Induction

With the data included in this study, a liver induction effect of around 1.5-fold and a gut induction effect of about 2-fold was estimated, depending on the dosing amounts (Fig. 5, C, F, and I). The result is consistent with a previous clinical study, in which bosentan increased the urinary excretion of 6 β -hydroxycortisol (an endogenous marker of CYP3A4 activity) 1.7-fold (Weber et al., 1999c). After 125 mg twice-daily oral dosing for 10 days, using the metabolic rate estimated in MCMC, bosentan F_g was calculated to be 0.630 (95% CI, 0.57, 0.68) during the first dose and 0.473 (95% CI, 0.41, 0.53) during the last dose. The ratio between two F_g values is 0.751 (95% CI, 0.70, 0.81). Overall, the estimated induction effect is higher in gut than that in liver, which is similar to previous results published for DDI between repaglinide and rifampin (Varma et al., 2013). Since tadalafil absolute bioavailability is unknown and we arbitrarily assume its $F_a F_g$ to be 1 in the absence of bosentan, the estimated $F_a F_g$ in the presence of bosentan (Table 3) is essentially the ratio of $F_a F_g$ between two conditions. Further assuming that its F_a is not affected by bosentan, its F_g in the presence of bosentan is reduced to 0.868 (95% CI, 0.70, 1.0) of the F_g value in the absence of bosentan. This is consistent with bosentan F_g changes described above. For warfarin, this ratio is around 1 (Table 3). This is consistent with the fact that warfarin bioavailability (and hence F_g) is nearly 1 (Holford, 1986) (i.e., warfarin has minimal gut metabolism).

We also generated in vitro CYP3A and CYP2B6 induction data using human hepatocytes (Table 4; Supplemental Fig. 2) to understand the prediction accuracy of the current in vitro tool. By measuring activity, the prediction from lot HH1025 was closest to the in vivo simulations, whereas the other two lots (i.e., HC7-4 and FOS) would overpredict in vivo induction (Fig. 6). By measuring mRNA, the assay overpredicts observed induction based on clinical data, which is consistent with another P450 inducer, rifampin (in-house data not shown).

BSEP, MRP, and NTCP Inhibition

With the predicted unbound liver tissue or plasma exposure and published IC_{50} values estimated from in vitro data, the model predicted moderate inhibition (up to 18%) for these transporters (Fig. 7).

Discussion

This study aimed to predict the liver concentration of a transporter substrate by leveraging a PBPK model that utilizes available clinical (systemic plasma concentration) data. The underlying mechanism for such a prediction is the conservation of mass: the total amount of the compound in systemic blood, liver, nonliver tissues, and the compound metabolized is equal to the dosed amount. In such a scenario, the dosed amount is known. The amount in systemic blood is based on measured

Fig. 5. (A–I) Simulated total systemic and liver tissue concentrations (A, D, and G), ratios between unbound liver tissue and unbound systemic plasma concentrations (B, E, and H), and induction effects of bosentan (C, F, and I). Red and blue curves in subplots (A), (D), and (G) represent systemic and liver concentrations, respectively. Red and blue curves in subplots (C), (F), and (I) represent induction effects in enterocytes and the liver, respectively. The solid lines and shaded areas represent the median and 95% CI of the simulations generated with parameter values identified in Markov chain Monte Carlo. BID, twice daily; QD, once daily.

plasma concentrations, the amount in nonliver tissue is predicted with *in silico* methods, and the amount metabolized can be calculated using a hepatic metabolic rate estimated from systemic data. As a result, the amount in the liver can be deduced. A precise and accurate “deduction” is based on three criteria. First, the model’s ability to accurately describe systemic data (amount in systemic blood) is a prerequisite for predicting liver concentration, which explains why we established this relatively complex PBPK model incorporating multiple nonlinear processes. Second, there must be sufficient data to enable confident estimation of hepatic metabolism. For certain compounds, their clinical data cannot satisfy this requirement as described previously (Li et al., 2016). With an MCMC approach, we have shown that bosentan metabolism can be precisely estimated from its clinical systemic data. Third, an accurate

description of distribution into nonliver tissues is critical, which is usually predicted by the *in silico* estimated K_{pu} values in human PBPK modeling. To understand how inaccurate nonliver K_{pu} (distribution into nonliver tissues) may affect liver K_{pu} estimation, we applied a scaler for nonliver K_{pu} at the value of 0.1 or 10 and we re-estimated liver K_{pu} . With a nonliver K_{pu} scaler of 0.1, the goodness of fit of the systemic data is about the same as that without using a scaler, and the liver K_{pu} is about 40. The latter is still within the CI of the liver K_{pu} without a nonliver K_{pu} scaler. On the other hand, with a nonliver K_{pu} scaler of 10, the goodness of fit is significantly worse (i.e., objective function value increased by 5-fold) and the liver K_{pu} cannot be confidently identified. We also tried to estimate a K_{pu} scaler by including it as another fitted parameter; however, this parameter cannot be precisely estimated. In a

TABLE 4
Median values and 95% CIs of optimized in vitro parameters ($E_{\max}$ and EC_{50}) describing P450 induction due to bosentan

Assay and Hepatocyte Lot ^a	$E_{\max}$	EC_{50}
<i>nM</i>		
CYP2B6 activity		
HC7-4	9.62 (8.4, 11)	1440 (1100, 1900)
HH1025	1.40 (1.1, 1.7)	
FOS	9.13 (7.9, 11)	
CYP3A4 activity		
HC7-4	11.0 (9.5, 13)	433 (320, 590)
HH1025	1.62 (1.3, 2.0)	
FOS	8.26 (7.2, 9.5)	
CYP2B6 mRNA		
HC7-4	11.1 (9.0, 14)	958 (600, 1500)
HH1025	11.2 (9.0, 14)	
FOS	3.89 (3.0, 5.0)	
CYP3A4 mRNA		
HC7-4	127 (110, 150)	1118 (860, 1500)
HH1025	72.9 (60, 89)	
FOS	29.9 (25, 36)	

^aHC7-4 and FOS are from male donors and HH1025 is from a female donor.

monkey study presented in part 2 of this work (Li et al., 2018), in which both systemic and liver exposure are determined experimentally, we can confidently estimate the nonliver Kp_u scalar as 1.47, which justifies a value of 1 in the present exercise.

Binding to bosentan’s pharmacology target may also affect distribution. The ratio, K_D , between ET k_{off} and k_{on} is estimated to be 4.30 (95% CI, 1.4, 11) nM, which is very close to experimentally determined values (Russell and Davenport, 1995; Bacon and Davenport, 1996; Gatfield et al., 2012). Although we set these parameters for ET binding, the optimization process may also use them to describe tissue distribution not explained by tissue Kp_u fixed at the in silico predicted values. The fact that the estimated K_D value is similar to experimentally determined values suggests that the nonliver Kp_u used in the model is likely accurate. Alternatively, if nonliver Kp_u was inaccurate, the model would likely incorrectly estimate ET K_D .

Bosentan is mostly metabolized by CYP3A with a minor contribution from CYP2C9 (70% vs. 10%; Supplemental Material). For the induction victim drugs, tadalafil is primarily metabolized by CYP3A (Wrishko et al., 2008), CYP3A contributes to (R)-warfarin clearance, and (S)-warfarin is largely metabolized by CYP2C9 (Weber et al., 1999a). To reduce the number of fitted parameters, we assume that different P450s share the same induction $E_{\max}$ and EC_{50} , based on the fact that bosentan induces P450 via pregnane X receptor-mediated mechanisms. It is difficult to validate this assumption without additional clinical data. Because the estimated hepatic induction effect is minimal, the specific values for each hepatic P450 are unlikely to significantly affect current simulations. For enterocyte induction in the gut, which is stronger than hepatic induction, we assume that induction is compound specific. Nonetheless, we also re-estimated liver Kp_{uu} after removing data from

Fig. 6. Simulated induction effect based on clinical data (green) and in vitro hepatocyte data (red, black, and blue). Red, blue, and black represent simulations based on hepatocyte lots HC7-4, HH1025, and FOS, respectively. The solid lines and shaded areas represent the median and 95% CI of the simulations generate with parameter values identified in Markov chain Monte Carlo.

Fig. 7. (A) Simulated inhibition of BSEP (black), MRP3 (green), and MRP4 (magenta) based on bosentan unbound liver tissue concentration. (B) Simulated inhibition of NTCP (cyan) based on bosentan unbound liver plasma concentration. The solid lines and shaded areas represent the median and 95% CI of the simulations generate with parameter values identified in Markov chain Monte Carlo. BID, twice daily.

victim drugs. Neither estimated parameter values nor $K_{p_{uu}}$ changed significantly. As a result, even if the future data show that the assumption made here for hepatic induction E_{max} and EC_{50} is incorrect, it should not significantly confound the liver simulations. It is worth noting that the simulated induction effect (Fig. 5, C, F, and I) is consistent with the results of a previous clinical study, in which an endogenous marker of CYP3A4 activity was monitored. In addition, estimated changes of F_g values due to induction are similar between bosentan and tadalafil. These results are consistent with the fact that bosentan and tadalafil are mostly metabolized by CYP3A.

An average of 10% transporter inhibition is predicted, but the PBPK model does not include bosentan metabolites, which may lead to more inhibition. However, determining how much inhibition is required to cause DILI is beyond the scope of this study.

To increase confidence in the simulations, the model was trained with data collected from several clinical studies. Intravenous bolus studies are generally excluded from first-in-human studies due to the cost of developing the formulation, safety concerns, and so forth. However, these studies may be a cost-effective way to understand liver exposure (i.e., exposure at the site of action) compared with other approaches such as PET, which can be difficult to interpret due to metabolite interference. We therefore recommend that investigators performing human intravenous bolus studies collect data in both distribution and terminal phases for future clinical trials to provide a greater understanding of liver exposure for compounds undergoing metabolism. With only oral data, there are additional fitted absorption parameters that may lead to overparameterization. Oral studies may be acceptable for compounds with known $F_a F_g$. Failure in collecting data in the terminal phase may add uncertainty in identifying hepatic metabolism and liver exposure. If parameters cannot be confidently estimated even with intravenous data,

Bayesian-based approaches may have to be used with the risk of using inaccurate priors. For compounds with minimal metabolism but extensive biliary excretion and enterohepatic recirculation, PBPK modeling is restricted by our limited understanding of elimination mechanisms, whereas PET studies may provide more straightforward results. For compounds with both metabolism and enterohepatic recirculation (e.g., UDP-glucuronosyltransferase substrates), we have not yet seen any clinical approach beneficial in understanding their liver exposures.

Yoshikado et al. (2017) reported that a generally accepted CYP3A inhibitor, itraconazole, did not significantly change bosentan systemic exposure in humans. The authors estimated *in vivo* CYP3A inhibition by itraconazole using orally administered midazolam as a victim compound (where plasma area under the curve ratio was 3.7) and assumed that itraconazole could inhibit bosentan metabolism to the same level. The fact that itraconazole did not change bosentan systemic exposure led the authors to conclude that the ratio of hepatic metabolism to passive diffusion was high, such that the change of metabolism within the liver was not reflected in systemic exposure. This conclusion contradicts our parameter estimation, where passive diffusion is fast so that CYP3A inhibition will change systemic exposure (simulation not shown). It worth noting that in the study by Yoshikado et al. (2017), itraconazole did not significantly change bosentan metabolite exposure either. It is possible that itraconazole cannot sufficiently inhibit bosentan metabolism under *in vivo* conditions; hence, it would not affect bosentan systemic exposure, independent of bosentan's permeability. In addition, a second CYP3A inhibitor, ketoconazole, has been shown to significantly alter bosentan systemic exposure (van Giersbergen et al., 2002b), which is consistent with our prediction.

In conclusion, using bosentan data, we have provided an example to demonstrate how to translate the systemic concentration of a hepatic transporter substrate into its liver exposure by leveraging a PBPK-based “deduction” method. The precision and accuracy of such a translation was also evaluated and discussed. As described, this new approach supports the determination of drug liver exposure in humans based on existing clinical data, as information regarding the exposure at the site of action is critical for hepatic transporter substrates when attempting to understand their pharmacodynamics, DDI, toxicity in the liver, and therapeutic index.

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Authorship Contributions

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