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ORIGINAL ARTICLE

Prediction of the Pharmacokinetics of Pravastatin as an OATP Substrate Using Plateable Human Hepatocytes With Human Plasma Data and PBPK Modeling

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Plateable human hepatocytes with human plasma were utilized to generate the uptake transporter kinetic data for pravastatin, an organic anion-transporting polypeptide (OATP) transporter substrate. The active hepatic uptake of pravastatin was determined with a J_{max} value of 134.4 pmol/min/million cells and K_m of 76.77 μM in plateable human hepatocytes with human plasma. The physiologically-based pharmacokinetic (PBPK) model with incorporation of these *in vitro* kinetic data successfully simulated the i.v. pharmacokinetic profile of pravastatin without applying scaling factor (the mean predicted area under the curve (AUC) is within 1.5-fold of the observed). Furthermore, the PBPK model also adequately described the oral plasma concentration-time profiles of pravastatin at different dose levels. The current investigation demonstrates an approach allowing us to build upon the translation of *in vitro* OATP uptake transporter data to *in vivo*, with a hope of utilizing the *in vitro* data for the prospective human pharmacokinetic (PK) prediction.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The PBPK models of pravastatin have been developed by various groups previously, and the OATP $Cl_{int,T}$ input was either from the *in vitro* measurement with a requirement of a scaling factor or back calculated from *in vivo* data. It demonstrates a hurdle to the utilization of the *in vitro* data for prospective PK prediction of a compound that is an OATP substrate by PBPK.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Is it possible to apply a PBPK model that incorporates the *in vitro* OATP hepatic uptake data generated in the developed system to directly predict the PK of pravastatin.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Incorporation of the OATP kinetic uptake data generated in plateable human hepatocytes with human plasma enabled the PBPK model to simulate the i.v. and oral PK profiles of pravastatin successfully without incorporating a scaling factor.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS

☑ It demonstrates an approach allowing us to build upon the translation of *in vitro* OATP uptake transporter data to *in vivo* for pravastatin. It invites additional studies on more OATP substrates, with a hope of eventually utilizing the *in vitro* data for the prospective human PK prediction.

Active hepatic uptake is a critical step in drug disposition that may impact the pharmacokinetic (PK) properties of certain drugs. As illustrated extensively by examples of statin family, it is important to understand the role of the active hepatic uptake in drug clearance and its subsequent impact on drug disposition in order to understand the relationship between the intracellular concentration and observed efficacy.[1] Organic anion-transporting polypeptides (OATPs) are well known hepatic uptake transporters, and many statins are known to be substrates of these transporters.

Pravastatin is known to be a hepatic OATP uptake transporter substrate. Based on the human mass balance study after i.v. administration, 60% of the total dose of pravastatin was found in the urine and 34% was recovered in the feces (mainly through the biliary clearance).[2] The fraction of oral dose of pravastatin absorbed is 34% and absolute bioavailability is 17%.[2] There is a small amount of metabolism mediated by P450 enzymes, and their contribution to total clearance is minimal, such that cytochrome P450 inhibitory drug interactions have no real effect on the PK of pravastatin.[3] Therefore, it is believed that the active hepatic uptake process plays an important role in pravastatin's clearance. The *in vitro* studies suggest that the hepatic uptake of pravastatin is mainly mediated by OATP1B1.[4]

The physiologically-based pharmacokinetic (PBPK) approach has demonstrated its value in predicting the dynamic concentration-time profile in the blood and tissues by combining the mechanistic understanding of the active hepatic uptake with the physiological, genetic, and demographic information. More importantly, PBPK provides a platform that allows testing of mechanistic hypotheses with respect to tissue concentrations in order to build pharmacokinetic/pharmacodynamic

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relationships for the active uptake substrates.[5,6] The Simcyp ® population-based ADME simulator is one of the commercial PBPK software available to facilitate the PK profile prediction for compounds, such as pravastatin, that has PK disposition involving the active hepatic uptake transporters.[7] The PBPK models of pravastatin have previously been developed by various groups[5,7–9] in order to predict the PK and transported mediated drug-drug interactions. Inclusion of OATP transporter kinetic parameters is critical in predicting the PK profile of pravastatin. In the Simcyp default compound profile, in order to describe the clinical data, the OATP $Cl_{int,T}$ input of hepatic uptake parameter for pravastatin was back calculated by fitting to the observed *in vivo* PK data. In the other studies,[5,7–9] the *in vitro* OATP $Cl_{int,T}$ data generated in the suspended hepatocytes and the sandwich cultured hepatocytes were used. Nevertheless, when the *in vitro* data were incorporated into the PBPK model, a scaling factor was found to be necessary in order to predict the observed pravastatin PK profile accurately. It is evident that a different scaling factor is needed for different OATP substrates despite the *in vitro* data being generated in the same laboratory.[8] This phenomenon demonstrates an important hurdle to the utilization of the *in vitro* data for prospective PK prediction of a new chemical entity (NCE) that is a substrate of OATP by PBPK modeling.

One of the approaches to overcome this challenge is to develop an *in vitro* model that can directly provide quantitative and translatable kinetic parameters for OATP substrates. Plateable human hepatocytes with human plasma are proposed as a promising *in vitro* tool for OATP transporter uptake assessment. Compared with the overexpressing cell lines, human hepatocytes provide an intact cell environment with relatively correct expression level of transporters, which mimics the physiological situation. In addition, compared to the medium without human plasma, the generated parameters in this system could inherently account for the *in vivo* impact of the binding activity on the active uptake process, including the plasma protein binding effect and potential interaction between plasma proteins and transporters. Ideally, if the *in vitro* generated active uptake parameters can successfully describe the PK of various OATP substrates when incorporated into the PBPK models, there will be higher confidence to utilize the *in vitro* data for prospective PK prediction for NCE as an OATP substrate.

In the present work, we applied the PBPK model that incorporates the hepatic uptake transporter data generated in the developed *in vitro* system (plateable human hepatocytes with human plasma) to directly predict the PK of pravastatin without applying a scaling factor. The performance of the PBPK model was assessed by simulating the i.v. PK of pravastatin and further verified by simulating the oral PK at different doses. Pravastatin was selected for this work because it has been studied by several laboratories using transporter data generated from different *in vitro* systems or by fitting of *in vivo* clinical data. The aim of this current work is to present an approach that could potentially improve our understanding of the translation of *in vitro* OATP transporter data to *in vivo* disposition. The limitations of the work and future studies will be discussed.

MATERIALS AND METHODS

Material

Pravastatin was purchased from Cayman Chemical Company (Ann Harbor, MI), and the rifampicin and tolbutamide were obtained from Sigma-Aldrich (St. Louis, MO). Cryopreserved human hepatocytes and collagen coated 24-well plates (CellAffix™) were obtained from A.P. Sciences (Columbia, MD). Universal cryopreserved cell recovery medium (UCRM™), hepatocyte induction medium (HIM™), modified human plasma medium (100% human plasma) (HPZ-A™), hepatocyte rinse medium (HRM™), and hepatocyte incubation medium (HQM™) were obtained from In Vitro ADMET Laboratories (IVAL, Columbia, MD).

Active hepatic uptake transporter studies in plateable human hepatocytes with human plasma

Cryopreserved plateable human hepatocytes from one donor (lot #HH1057), a 33-year-old caucasian female, were thawed, and recovered using universal cryopreserved cell recovery medium. The cells were centrifuged at 100 × g for 10 minutes and, after viability determination by the trypan dye exclusion method, the cells were seeded in a collagen coated 24-well plate at a density of 350,000 cells/well (500 μL of 0.7 × 10^6 cells/mL cell suspension) using hepatocyte induction medium. The cells were incubated in a humidified 95% balanced air/5% CO_2 atmosphere in a 37°C incubator for 4 hours to facilitate the attachment. Following the attachment, the media was removed and the monolayer was washed to remove any unattached cells. Pravastatin at 10 concentrations (400, 300, 200, 100, 33.3, 11.1, 3.7, 1.2, 0.4, and 0.14 μM) was tested in modified human plasma medium, either alone or in the presence of uptake transporter inhibitor rifampicin at 125 μM. The uptake was initiated by the addition of pravastatin either alone or in combination with rifampicin. The reactions were terminated at 0 minutes and 3 minutes by washing the cells 4 times with ice-cold rinse media-HRM™ and 3 times with ice-cold incubation media-HQM. The incubation time of 3 minutes was selected based on the linear increase of the active uptake without the interference of passive uptake process. After rinse, the cells were lysed in HQM:organic mixture (acetonitrile and methanol in 3:1 ratio) containing internal standard (10 nM tolbutamide), centrifuged, and the supernatants were subjected to liquid chromatography tandem mass spectrometry analysis.

Bioanalysis of pravastatin in plated human hepatocytes

The liquid chromatography tandem mass spectrometry analysis was carried out with a Kinetex C18 column (Phenomenex, Torrance, CA) particle size 2.6 μM, 50 mm × 3 mm using the Acquity UPLC system coupled with an Applied Biosystems API5000 mass spectrometer. The separation was achieved by using a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as mobile phases. The total mobile phase was delivered at 0.6 mL/min according to an elution program that started at 5% of B for 0.37 minutes and ramped to 95% of B over 1.13 minutes. The percentage of B was held at 95% for 0.2 minutes and returned to the initial 5% of B at 0.1 minutes.

Table 1 Physiologically-based pharmacokinetic model input parameters for pravastatin

Input parameters	Value	Source	Input parameters	Value	Source
Physicochemical properties			**Transporter**		
MW	424.5	Default	Intestine		
LogP	2.2	Default	CL_{int}, MRP2 (μL/min/10^6 cells)	1.2	Default
Compound type	Monoprotic acid	Default	Scaling factor	0.18	Default
pKa	4.55	Default	Liver		
Fraction unbound	0.485	Default	CL_{PD} (mL/min/10^6 cells)	0.0001	Default
Blood/plasma ratio	0.556	Default	$J_{max,\ OATP}$ (pmol/min/10^6 cells)	134.4	Current investigation
Absorption			$K_{m,\ OATP}$ (uM)	76.77	Current investigation
Model	ADAM		$f_{u,inc}$	0.485	Same as fu,p
Intrinsic transcellular permeability $P_{tran,0}$ (10^{-6} cm/s)	100	Default model fitted as 800	CL_{int}, MRP2 (μL/min/10^6 cells)	1.2	Default
Input form	Solution		Scaling factor	0.18	Default
Distribution			Kidney (OAT3/MATEs)		
Model	Full PBPK		CL_{int},T (μL/min/10^6 cells)	180/180	Default
Kp scalar	1		Scaling factor	1/1	Default
Elimination					
Additional HLM CL_{int} (μL/min/mg protein)	4	Default			

ADAM, advanced dissolution, absorption, and metabolism; CL_{int}, intrinsic clearance; CL_{int},T, transporter intrinsic clearance; CL_{PD}, passive diffusion clearance; $f_{u,inc}$, unbound fraction in the incubation; $f_{u,p}$, unbound fraction in human plasma; HLM, human liver microsome; J_{max}, maximum rate of active uptake; Km, substrate concentration at 50% of maximum rate of active uptake; LogP, partition coefficient between aqueous and lipophilic phase; MATE, multidrug and toxin extrusion; MRP, multidrug resistance protein; MW, molecular weight; OATP, organic anion-transporting polypeptide; OAT3, organic anion transporter-3; PBPK, physiologically-based pharmacokinetic.

The column was allowed to re-equilibrate for 1.3 minutes before the next injection.

The samples were analyzed in the negative ionization mode. The m/z for pravastatin and tolbutamide were 423.192 → 321.10 and 269.08 → 170.00, respectively. The lower and upper limits of quantification were 0.23 nM and 2 μM of pravastatin. Declustering potential was −160 V and collision energy was −22 V for pravastatin. Declustering potential was −115 V and collision energy was −26 V for the IS.

Modeling of the active hepatic uptake transporter kinetic parameters J_{max} and Km of pravastatin

The following parameters were calculated using the measured pravastatin concentration.

Absolute intracellular pravastatin concentration. Mean of intracellular concentration at time 0 minutes for each tested concentration (A) was calculated, which was then subtracted from the corresponding measurement (B) at 3 minutes (X = B-A). Mean of intracellular concentration in the presence of rifampicin at time 0 minutes for each tested concentration (C) was calculated, which was then subtracted from the corresponding measurement (D) at 3 minutes (Y = D-C).

Net pravastatin uptake calculation: N = X-Y. The calculated N (converted in the unit as pmol/min/million cells) and nominal concentrations were used for fitting by the Michaelis-Menten kinetic model (GraphPad Prism 7), and the J_{max} and the apparent K_m were estimated.

Clinical pharmacokinetic data

A total of nine PK profiles of pravastatin from a literature search were identified and all utilized in the current PBPK model development and verification. Only one i.v. study was reported in the literature.[2] Eight single oral dose PKs with different doses were reported in the literature: 0.0372 mg,[10] 19.2 mg,[2] 20 mg,[11,12] 40 mg,[13–15] and 60 mg.[16] Clinical studies used for the PBPK model development: i.v. dose of 9.4 mg[2] and oral dose of 40 mg[13–15]; clinical studies used for the PBPK model verification: oral dose of 0.0372 mg,[10] 19.2 mg,[2] 20 mg,[11,12] and 60 mg.[16]

Pravastatin PBPK model

Simcyp, version 15 (Certara, Princeton, NJ) PBPK model for pravastatin was used in this investigation. The input parameters for pravastatin model are summarized in **Table 1**. Information that has been previously disclosed in the Simcyp compound profile of pravastatin is indicated in **Table 1**. The basic model structure and details of model modification are described below.

Distribution

In the default compound profile, full PBPK with perfusion limited distribution model was used. The volume of distribution at steady state was calculated by the tissue partition coefficient using the equation described by the Rodgers *et al.*[17,18] and the Rodgers & Rowland[19,20] methods considering the rapid equilibrium between blood and tissues.

Clearance

In the default compound profile, the total clearance is assigned to the biliary clearance (40%), renal clearance

(47%), and the metabolism clearance (13%) based on the clinical data and mass balance study data.[2] The biliary clearance comprises (1) passive diffusion and OATP hepatic uptake transporter on the sinusoidal membrane and (2) multiple drug resistance-associated protein 2 (MRP2) efflux transporter on the canalicular membrane.[21–23] For the current investigation, the kinetic parameters of OATP uptake transporter generated in the plateable human hepatocytes with human plasma were used to replace the default model input in the model. Because the incubation medium is 100% human plasma, the fu,inc was assumed to be the same as the fu,p 0.485 in the model input. As the *in vitro* study is performed in human hepatocytes, it is challenging to distinguish the contribution between OATP1B1 and OATP1B3 on pravastatin. Therefore, the kinetic parameters are assigned to the OATP1B1 because this is the dominant transporter.[4] No additional scaling factor was applied for the prediction of uptake by the OATP transporter. The input for passive diffusion is 0.0001 mL/min/10^6 cells determined in sandwich culture hepatocytes.[8] The canalicular efflux by MRP2 was described using a measured $Cl_{int,T}$ (1.2 μL/min/10^6 cells) in sandwich culture hepatocytes[8] with a scaling factor of 0.18.[9]

The mechanistic kidney model was used to recover the observed renal clearance (26.6 L/h). The basal uptake was assigned to OAT3[24,25] and MATE (used as surrogate for an unidentified renal apical efflux transporter) with $Cl_{int,T}$ of 180 μL/min/10^6 cells for each transporter obtained from model fitting to the clinical PK data. An input of human liver microsome clearance (4 μL/min/mg protein) was used in the model as back calculation to account for the 13% metabolism clearance.

Absorption

In the default Simcyp compound profile, the absorption was predicted using the advanced dissolution, absorption, and metabolism (ADAM) model with pravastatin administered as a solution. A $Cl_{int,T}$ (1.2 μL/min/cm^2) was used for the intestinal MRP2 with the scaling factor of 0.18.[8] The MechPeff model was used to predict the permeability.[26] The value of intrinsic transcellular permeability is used for $P_{eff,man}$ prediction for the regions of the intestine, and it was updated in the current investigation as 100×10^{-6} cm/s in order to reflect the reported bioavailability (observed Fa and F as 0.34 and 0.17, and predicted Fa and F as 0.26 and 0.23, respectively).

PBPK model simulation

The prediction of distribution and clearance of pravastatin was evaluated by simulating the i.v. PK profile of pravastatin.[2] Following the reported clinical study design, a single dose of 9.4 mg pravastatin was given as i.v. bolus over 2 minutes to healthy subjects with a body weight of 70 kg, age ranging from 21 to 39 years, and 50% as women. The simulation was conducted in a virtual population consisting of 10 trials of 8 individuals. The prediction of absorption of pravastatin was assessed by simulating the oral PK profile of pravastatin. The PK profiles of the 40 mg[13–15] dose group were chosen because the reported concentration-time profile covers a longer time (up to 12 hours) in comparison with other doses.

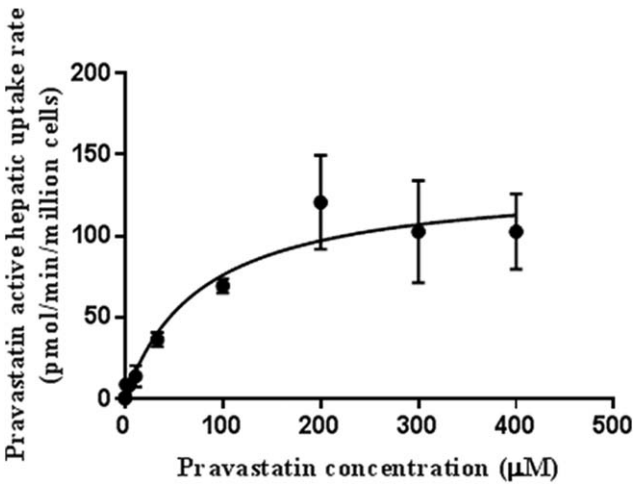

Figure 1 The rate of the hepatic active uptake against concentration of pravastatin in plateable human hepatocytes with human plasma ($N = 3$).

The model was further verified with additional oral PK data from doses of 0.0372 mg,[10] 19.2 mg,[2] 20 mg,[11,12] and 60 mg.[16] The simulation of oral PK was conducted using virtual population of healthy subjects (10 trials of 23 individuals per trial) with body weight of 70 kg, age ranging from 18 to 25 years, with 53% women.

RESULTS

Active hepatic uptake of pravastatin by OATP transporter in plateable human hepatocytes with human plasma

Considering the plasma protein binding (fu,p 0.485) of pravastatin, to obtain the full saturation kinetic curve in plateable human hepatocytes with human plasma, a wide range of pravastatin concentration, ranging from 0.14–400 μM, were tested. In order to examine the active uptake process more closely, a high concentration of rifampicin (125 μM) was used in the current study with the intention to inhibit the OATP uptake process. The concentration of pravastatin associated with the cell was measured and the absolute intracellular pravastatin concentration was calculated (details described in the Methods section).The net active uptake of pravastatin is extracted from the incubation without and with rifampicin at 0 and 3 minutes across the tested concentrations of pravastatin. The J_{max} and the apparent K_m for pravastatin were determined as 134.4 ± 14.3 pmol/min/million cells and 76.77 ± 27.4 μM, respectively (**Figure 1**).

Prediction of pravastatin PK using the PBPK model

When the *in vitro* OATP transporter kinetic data were incorporated into the PBPK model, the model simulation captured the observed i.v. PK profile adequately (**Figure 2a**), without applying any scaling factor on the measured kinetic data from the plateable human hepatocytes with human plasma. The predicted PK profile by the current model captured the shape of the observed PK profile well, particularly

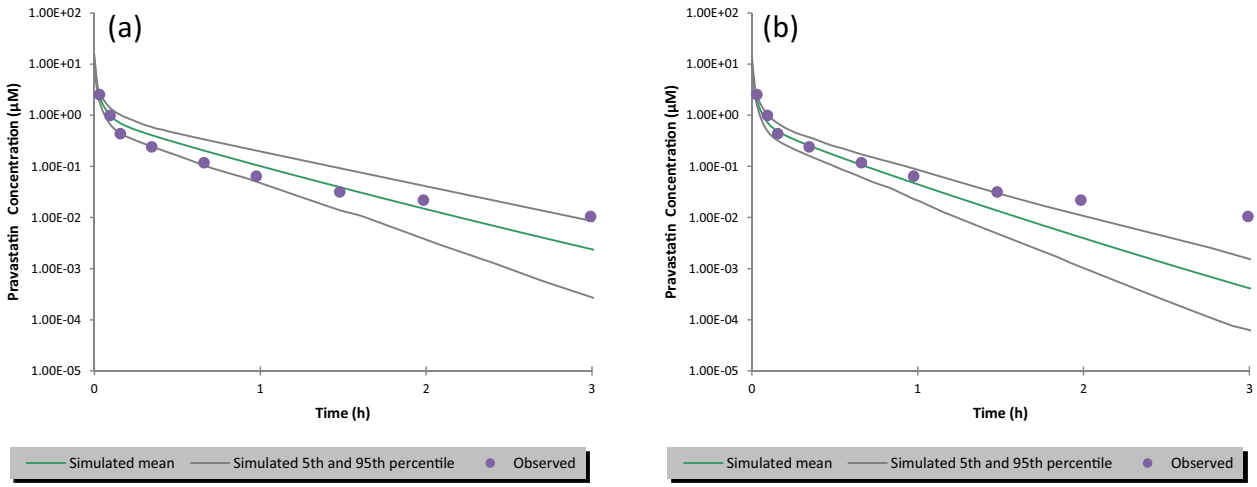

Figure 2 Simulated vs. observed plasma concentration-time profiles of pravastatin after an i.v. bolus dose of 9.4 mg over 2 minutes. The dots are the observed clinical data. The simulated mean is the mean of 80 individuals (10 trials of 8 subjects per trial); the 5th and 95th percentile for 80 individuals simulated represents the 5th and 95th highest concentrations from the ranked concentrations. (**a**) The simulation using the parameters listed in **Table 1**; (**b**) the simulation using the default profile in Simcyp, the input is the same as listed in **Table 1** except the $CL_{int,T}$ for organic anion-transporting polypeptide (OATP)1B1 and OATP1B3 was set to be 14.057 and 1.343 uL/min/million cells.

on the third distribution phase. The mean predicted pravastatin AUC (0.60 μM.hr) using the current model directly incorporating the *in vitro* kinetic data is within 1.5-fold of that predicted (0.43 μM.hr) using the default input of $CL_{int,T}$ that was back calculated by fitting to the observed *in vivo* PK data (0.4 μM.hr; **Figure 2b**).

When verifying the model's performance in simulating oral PK for pravastatin, the value of clearance and volume of distribution at steady state defined using the i.v. model was kept unchanged. The default model overpredicted Fa (0.76) and F (0.67). By modifying the intrinsic transcellular permeability, the model simulation provided values of Fa (0.26) and F (0.23) that closest represent the reported clinical data, Fa (34%) and F (18%).[2] Thereafter, the PBPK model simulation captured the oral pravastatin PK profile from 0.0372 mg up to 60 mg doses of pravastatin (**Figure 3a-e**). The predicted and observed peak plasma concentration (C_{max}) and AUC are shown in **Supplementary Table S2**. Although the model predicted mean C_{max} and AUC are within twofold of the observed data, a trend of underprediction of C_{max} was observed. Further refinement of the absorption model and/or investigation of variability between predicted and observed data are necessary when more clinical data become available.

DISCUSSION

Pravastatin is well known as an *in vivo* OATP substrate, and there are several pioneer investigations on the PBPK work for pravastatin.[5,7–9] The platform provided by a software company, such as Simcyp, provided an opportunity for researchers to test and generate hypothesis via simulations as well as to further improve models as science evolves. In a recently published Simcyp compound profile[7], the pravastatin PBPK model was built using a combination of the top-down and bottom-up approach. To capture the observed pravastatin PK profile, the $CL_{int,T}$ describing the OATP hepatic uptake were obtained through back calculation from *in vivo* data. For the other investigations, different *in vitro* assays have been explored to quantitatively assess the OATP uptake process for pravastatin. In the human suspended hepatocytes with an oil-spin method, the $CL_{int,T}$ was determined as 4.5 and 0.7 μL/min/million cells at 1 μM and 100 μM of pravastatin.[5] The scaling factor obtained in rats between *in vitro* hepatic uptake and *in vivo* observation was applied to the model in order to predict the observed human PK. In the sandwich culture hepatocytes, the $CL_{int,T}$ was determined to be 1.9 μL/min/million cell at 1 μM of pravastatin.[8] When these *in vitro* data were directly used in the PBPK model, it failed to capture the curve of the time concentration profile of pravastatin administered intravenously.[8,9] Therefore, a scaling factor was applied in the model in order to fit the observed data. In addition, when multiple OATP substrates were tested in the same *in vitro* system, different scaling factors were reported for each substrate. It seems to be challenging to use such information for the prospective prediction of hepatic uptake by OATP for a NCE. Currently, a mean of the scaling factors were recommended to apply for an NCE, and the prediction outcome using a mean scaling factor is deemed to be further investigated.

Although overprediction of pravastatin concentration is the main observation reported previously when *in vitro* data were incorporated in a PBPK model without use of a scaling factor,[8,9] the current PBPK model with the hepatic uptake kinetic data generated from plateable human hepatocytes with human plasma is able to reasonably predict pravastatin PK without applying any scaling factor. In our approach, a systematic investigation of optimal experiment conditions for plateable human hepatocytes with human plasma assay was conducted before finalizing the study

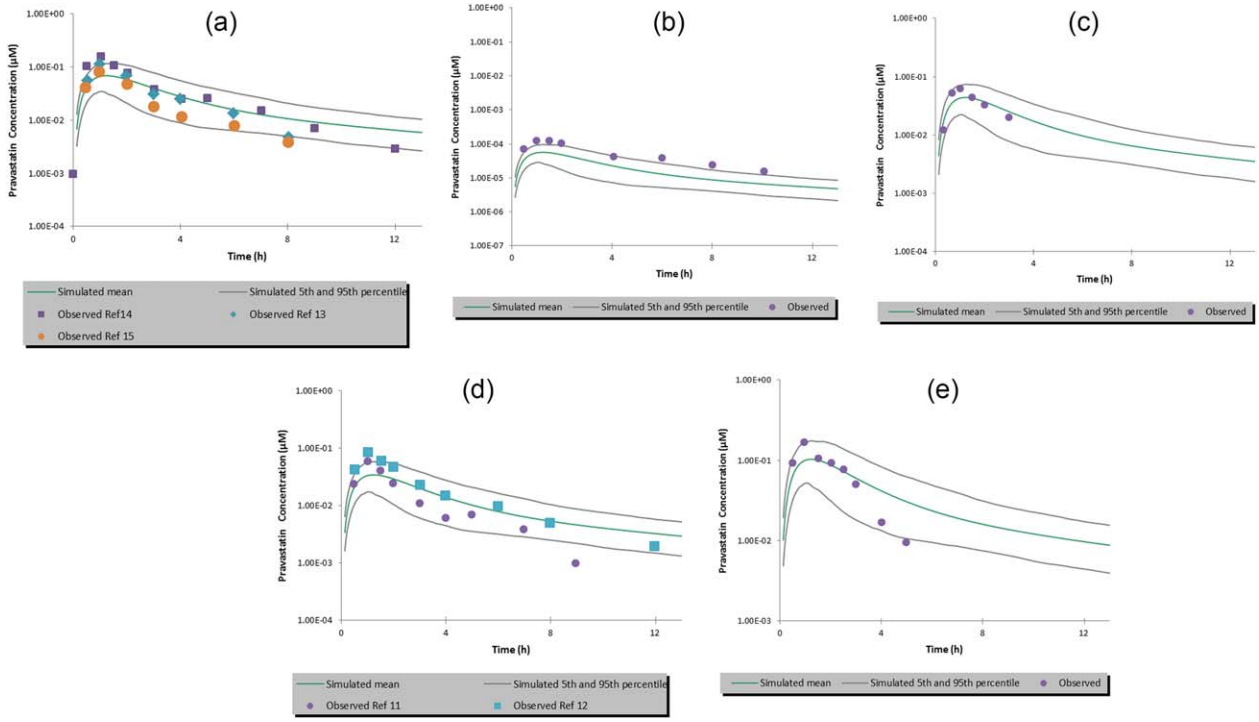

Figure 3 Simulated vs. observed plasma concentration-time profiles of pravastatin after a single oral dose of (**a**) 40 mg; (**b**) 0.0372 mg; (**c**) 18.23 mg; (**d**) 20 mg, and (**e**) 60 mg. The dots are the observed clinical data. The simulated mean is the mean of 230 individuals (10 trials of 23 subjects per trial); the 5th and 95th percentiles for 230 individuals simulated represents the 5th and 95th highest concentrations from the ranked concentrations. The input parameters used in the simulation are listed in **Table 1**.

design. This optimization includes: the duration for the cell attachment, the uptake assay time point, the inclusion of the rifampicin, the final wash medium, and evaluation of the usefulness of monitoring medium concentration. Although there is no clear guidance on the uptake rate required, the initial screen and selection of the hepatocyte lot is critical for success. The expression level of OATP1B1 and OATP1B3 (18.3 and 1.7 fmol/million cells) in the hepatocyte lot used in the study is within the range of the literature reported level (3.4–23.2 for OATP1B1 and 1.5–6.5 for OATP1B3).[27–30] Because pravastatin undergoes only minimal metabolism and the incubation time was short, metabolism in hepatocyte is likely not an issue in this study. However, for drugs with considerable metabolism, a longer incubation time may be needed in order to monitor metabolite formation in addition to a short time point to characterize the active uptake process. Both parent and metabolite information needs to be integrated into the PBPK model. In the current investigation, instead of $Cl_{int,T}$, the hepatic uptake kinetic data, J_{max}, and apparent K_m (134.4 ± 14.34 pmol/min/million cells and 76.77 ± 27.43 μM, respectively) of pravastatin were characterized in plateable human hepatocytes with human plasma and used in the PBPK modeling. Sufficient concentrations of pravastatin were tested *in vitro* to allow generation of the reliable uptake kinetic parameters, and the inclusion of the OATP inhibitor rifampicin enables the *in vitro* data to reflect the active uptake process from the interference of the passive permeability. In general, the use of uptake transporter kinetic data, such as *in vitro* K_m, enables the prediction of *in vivo* uptake clearance to be sensitive to the *in vivo* substrate concentration, which is more critical for substrates that have apparently nonlinear PK. This is important because the substrate concentration in hepatic inlet is the functional driver for the uptake transporters in the liver and this value usually changes dramatically with time. Moreover, when the *in vitro* transporter kinetic data were used for the PK prediction, the apparent K_m was corrected with human plasma protein binding to combine with the free concentration as the functional systemic concentration utilized in the PBPK model. Having 100% human plasma as incubation medium may account for the *in vivo* impact of the human plasma protein on the active uptake process. This includes the impact of plasma protein binding on the active uptake parameter assessment, the potential interaction between plasma proteins and transporters at the functional level, and the interplay between the rate of the plasma protein binding and the rate of the active uptake process. The inclusion of human plasma in the incubation may benefit more the substrates with the high plasma protein binding by limiting the introduction of uncertainty associated with binding into the model prediction. Considering that pravastatin is not highly bound to the human plasma proteins ($f_{u,p} = 0.485$), we also generated *in vitro* uptake kinetic data in plated human hepatocyte with the induction medium (not human plasma) and expected a similar prediction on pravastatin PK. Indeed, when the *in vitro* data (J_{max} 28.49 ± 3.11 pmol/min/million cells and K_m 11.72 ± 6.83 μM) corrected

for hepatocyte binding of pravastatin ($f_{u,inc}$ 0.96)[31] was incorporated into the same model, the simulations were able to capture the observed pravastatin PK without applying scaling factors (**Supplementary Figure S1**).

Although the current findings are encouraging, there are limitations and future studies are necessary in order to apply the approach broadly to all OATP substrates. First, it is known that the *in vitro* transporter data generated from different laboratories could be substantially different due to hepatocyte lots and experiment conditions used. Therefore, caution should be used when applying this *in vitro* approach across different laboratories. To increase the confidence in the prospective prediction, the *in vitro-in vivo* translational relationship should be assessed and established under each laboratory's condition using a relevant known transporter substrate. It is anticipated that the relationship established could be different from one laboratory to another. Second, to further demonstrate the superiority of plasma medium over the traditional medium for the quantitative prediction, investigation using the same approach with more OATP substrates, which have different extent of plasma protein binding, is needed. Third, to broadly apply the current approach for prospective prediction, the investigation of the PBPK model's performance in predicting OATP transporter-mediated drug-drug interactions (**Supplementary Table S3**) using transporter inhibition data generated from plateable human hepatocytes with human plasma and the performance in simulating the pravastatin PK for clinical genetic polymorphism scenarios using the uptake data generated in overexpressed cell lines for OATP1B1 and OATP1B3 are necessary. All these could further improve our understanding of the *in vitro* to *in vivo* translation. Furthermore, a better understanding of other model parameters is also important for overall accurate prediction of PK for the OATP transporter substrates. The quantitative translation of *in vitro* transporter data variability to the predicted *in vivo* transporter clearance needs to be further studied once more clinical data become available.

It is fully recognized that even though the complete mechanism is yet to be understood, the present study result with pravastatin provides a first step toward bridging the gaps to the unknown. With more OATP substrates tested in this same way, the approach will allow us to build upon the translation of *in vitro* OATP uptake transporter data to *in vivo* disposition with a hope of utilizing the *in vitro* data for the prospective human PK prediction.

In conclusion, the OATP active hepatic uptake kinetic data generated in plateable human hepatocytes with human plasma enabled successful PBPK model simulation of pravastatin PK profile without applying a scaling factor.

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Conflict of Interest. J.M., M.W., C.E.C.A.H., and Y.C. are employees of Genentech, a member of the Roche Group and also hold stock/shares in Genentech, a member of the Roche Group. U.D. was an employee of Vitro ADMET Laboratories Inc. A.P.L. is an employee of In Vitro ADMET Laboratories and holds shares in In Vitro ADMET Laboratories.

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