Physiologically Based Pharmacokinetic Model of the CYP2D6 Probe Atomoxetine: Extrapolation to Special Populations and Drug-Drug Interactions[§]

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ABSTRACT

Physiologically based pharmacokinetic (PBPK) modeling of drug disposition and drug-drug interactions (DDIs) has become a key component of drug development. PBPK modeling has also been considered as an approach to predict drug disposition in special populations. However, whether models developed and validated in healthy populations can be extrapolated to special populations is not well established. The goal of this study was to determine whether a drug-specific PBPK model validated using healthy populations could be used to predict drug disposition in specific populations and in organ impairment patients. A full PBPK model of atomoxetine was developed using a training set of pharmacokinetic (PK) data from CYP2D6 genotyped individuals. The model was validated using drug-specific acceptance criteria and a test set of 14 healthy subject PK studies.

Population PBPK models were then challenged by simulating the effects of ethnicity, DDIs, pediatrics, and renal and hepatic impairment on atomoxetine PK. Atomoxetine disposition was successfully predicted in 100% of healthy subject studies, 88% of studies in Asians, 79% of DDI studies, and 100% of pediatric studies. However, the atomoxetine area under the plasma concentration versus time curve (AUC) was overpredicted by 3- to 4-fold in end stage renal disease and hepatic impairment. The results show that validated PBPK models can be extrapolated to different ethnicities, DDIs, and pediatrics but not to renal and hepatic impairment patients, likely due to incomplete understanding of the physiologic changes in these conditions. These results show that systematic modeling efforts can be used to further refine population models to improve the predictive value in this area.

Introduction

Physiologically based pharmacokinetic (PBPK) models integrate population-specific physiologic parameters with drug-specific physicochemical and pharmacokinetics (PK) information to describe and predict drug disposition in various populations (Jones et al., 2009). PBPK modeling has become widespread in drug development, with different model development and acceptance strategies employed at different stages of drug development (Jones et al., 2015). Due to their mechanistic nature and the possibility of integrating population-specific physiologic changes, PBPK models may allow prediction of drug disposition in challenging clinical situations such as renal impairment (RI) and hepatic impairment (HI) patients, pediatric populations, and pregnant women prior to clinical studies. The confidence in the use of PBPK modelling in preclinical and clinical PK predictions and in drug-drug interaction (DDI) predictions for drugs mainly cleared by cytochrome P450s (P450s) is high (Jones et al., 2015), and the use of PBPK modeling to predict standard drug

disposition has been widely recommended (Committee for Medicinal Products for Human Use [CHMP], 2005; Rowland et al., 2011; Center for Drug Evaluation and Research, 2012; Huang and Rowland, 2012; Huang et al., 2013). However, while population models for HI, RI, and pediatrics have been incorporated into some of the PBPK simulation platforms (Edginton et al., 2006; Johnson et al., 2006, 2010; Edginton and Willmann, 2008; Rowland Yeo et al., 2011), confidence in PBPK modeling in situations involving ethnic variations, pediatrics, renal and hepatic insufficiency, and active transport is low or moderate (Jones et al., 2015; Wagner et al., 2015). Overall, it has been reported that the predictive performance of PBPK models in organ impairment populations still remains to be demonstrated, and additional research is needed on system components in this area (Wagner et al., 2015). Limited experience in other specific populations has been stated to prevent drawing conclusions on the predictive performance of PBPK modeling (Wagner et al., 2015). Critically, systematic studies to delineate the underlying reasons why PBPK modeling approaches fail to predict drug disposition in specific clinical scenarios are lacking. Generally, this could be due to poor drug models, inaccurate or incomplete population models, or low-quality and/or inaccurate clinical data. Distinguishing between these causes is particularly challenging since each drug has its own inherent PK variability, and therefore the commonly used *n*-fold metric to assess model performance may be too stringent for some drugs and too tolerant for others in evaluating model performance (Abduljalil et al., 2014), regardless of population and drug model quality. The aim

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ABBREVIATIONS: AUC, area under the plasma concentration versus time curve; CI, confidence interval; CL_{int}, intrinsic clearance; DDI, drug-drug interaction; EM, extensive metabolizer; ESRD, end stage renal disease; HI, hepatic impairment; IM, intermediate metabolizer; NDA, new drug application; CYP, cytochrome P450; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetics; PM, poor metabolizer; RI, renal impairment; V_{ss}, Volume of distribution at steady state.

of this study was to test whether a drug PBPK model validated for healthy volunteers could be extrapolated to special populations with current knowledge of the system components. A recently proposed, statistically rigorous model acceptance criterion (Abduljalil et al., 2014) was used to evaluate model performance, to account for drug-specific variability in PK data, and to avoid bias from variable quality of clinical data. A rigorous training/validation/extrapolation workflow (Fig. 1) was employed to delineate drug- and population-specific factors affecting model performance. Atomoxetine was used as the model compound since it is a Food and Drug Administration (FDA) recognized, well characterized, and a sensitive CYP2D6 probe substrate. Detailed in vitro data on metabolic pathways of atomoxetine and the inhibition of CYPs by atomoxetine is available (Ring et al., 2002; Sauer et al., 2004; Shen et al., 2007), as well as extensive in vivo intravenous and oral dosing PK data including absolute bioavailability in CYP2D6 genotyped populations (Center for Drug Evaluation and Research, 2002; Sauer et al., 2003). In addition, atomoxetine PK has been well characterized in pediatric populations (Center for Drug Evaluation and Research, 2002; Brown et al., 2016), in several different ethnic groups including Japanese and Chinese populations with different CYP2D6 genotypes (Center for Drug Evaluation and Research, 2002; Cui et al., 2007), and in HI populations and end stage renal disease (ESRD) (Center for Drug Evaluation and Research, 2002; Chalon et al., 2003). Since detailed PK parameters for atomoxetine are available in all of the aforementioned populations and in thorough DDI studies with atomoxetine as a precipitant or object drug

(Center for Drug Evaluation and Research, 2002; Sauer et al., 2004; Todor et al., 2015), atomoxetine provides an ideal model substrate to evaluate comprehensive model validation and extrapolation from healthy adult populations to various special populations, and for analysis of population and drug model performance.

Materials and Methods

Data Sources and Subject Demographics. Human clinical PK data available for atomoxetine was collected from the University of Washington Drug Interaction Database (<http://www.druginteractioninfo.org>), National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov/pubmed>), and new drug application (NDA) database (<http://www.fda.gov/Drugs/InformationOnDrugs>), accessed on January 1, 2016. Search keywords included “atomoxetine” in the NDA and University of Washington Drug Interaction Database, and “atomoxetine AND pharmacokinetics” in the National Center for Biotechnology Information database. In total, 88 documents were identified that contained information on atomoxetine. Case reports and studies not reporting PK data were excluded, leaving 10 documents with relevant PK data (Belle et al., 2002; Center for Drug Evaluation and Research, 2002; Chalon et al., 2003; Sauer et al., 2003, 2004; Cui et al., 2007; Matsui et al., 2012; Choi et al., 2014; Todor et al., 2015; Brown et al., 2016). The majority of the identified human PK data was originally reported in the Strattera NDA submission package and later published (Belle et al., 2002; Chalon et al., 2003; Sauer et al., 2004; Matsui et al., 2012). The trials included in the present analysis, and the subject demographics for each trial are summarized in Supplemental Table 1.

Model Acceptance Criterion. For assessment of model performance, drug-specific model acceptance criteria based on variability in observed human PK data

Fig. 1. The workflow of developing and validating the atomoxetine full-PBPK model and extrapolating the validated model to predict atomoxetine disposition in different ethnicities, special populations, and DDIs.

for atomoxetine were calculated using recently published methods (Abduljalil et al., 2014) according to the following equations:

$$\sigma = \sqrt{\ln \left[\left(\frac{CV\%}{100} \right)^2 + 1 \right]} \quad (1)$$

$$A\bar{x} = \exp \left[\ln(\bar{x}) + 4.26 \frac{\sigma}{\sqrt{N}} \right] \quad (2)$$

$$B\bar{x} = \exp \left[\ln(\bar{x}) - 4.26 \frac{\sigma}{\sqrt{N}} \right] \quad (3)$$

where CV% represents the observed mean of the coefficient of variation of the area under the curve (AUC) or the $C_{\max}$ value from all of the identified PK trials; σ is the calculated variability of a given PK parameter in the population; $\bar{x}$ is the observed mean AUC or $C_{\max}$ value; and N is the mean number of subjects in the clinical studies. The calculated values of A and B are the upper and lower boundaries for acceptable fold error, respectively. The PK studies used for calculation of A and B for AUC and $C_{\max}$ included studies in CYP2D6 extensive metabolizers (EMs) and poor metabolizers (PMs) as summarized in Supplemental Table 2. The calculated acceptance criterion ranges were 0.56- to 1.77-fold and 0.74- to 1.35-fold for the AUC values in the EM and PM populations, respectively, and 0.76- to 1.32-fold and 0.75- to 1.34-fold for the $C_{\max}$ values in the EM and PM populations, respectively (Supplemental Table 3). Each simulated PK study was compared with the observed values and the results were ranked as acceptable or unacceptable based on whether the mean simulated value was within the acceptance criteria.

PBPK Parameter Input and Model Development. The atomoxetine full PBPK model was developed using both top-down and bottom-up approaches and Simcyp Population-Based Pharmacokinetic Simulator version 14 (Simcyp Limited, Certara, Sheffield, United Kingdom) (Jamei et al., 2009). The physicochemical properties and drug-specific in vivo PK characteristics were collected from the atomoxetine product label (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021411s0351bl.pdf) and the NDA submission file (Center for Drug Evaluation and Research, 2002). The fraction absorbed (F_a) for atomoxetine was estimated as 0.96 based on the recovery of atomoxetine and its metabolites in urine following intravenous and oral dosing in CYP2D6 PM subjects (Sauer et al., 2003). The blood-to-plasma ratio and Q_{gut} (Yang et al., 2007), a hybrid term including both villous blood flow and permeability through enterocyte membrane, were predicted in Simcyp.

The fraction of atomoxetine clearance by CYP2D6 ($f_{m,2D6}$) in the CYP2D6 EM population was estimated as 87.6% based on the difference in clearances in EM and PM populations using the following equation (Ito et al., 2005):

$$f_{m,2D6} = 1 - \frac{AUC_{EM} D_{PM}}{D_{EM} AUC_{PM}} \quad (4)$$

The final $f_{m,2D6}$ value was calculated as a mean value of $f_{m,2D6}$ values calculated from three pairs of genotyped CYP2D6 EM and PM in vivo PK studies in the NDA as shown in Supplemental Table 4. The remaining hepatic clearance was attributed to CYP2C19, based on a human in vivo study in genotyped individuals assessing the role of CYP2C19 in atomoxetine metabolism (Choi et al., 2014).

The intrinsic clearance (CL_{int}) values of atomoxetine by CYP2D6 ($CL_{\text{int,CYP2D6}}$) and CYP2C19 ($CL_{\text{int,CYP2C19}}$) were calculated using the Simcyp retrograde calculator based on the estimated $f_{m,2D6}$ and systemic atomoxetine clearance measured after intravenous administration to CYP2D6 EM subjects (Center for Drug Evaluation and Research, 2002). Based on this approach the $CL_{\text{int,CYP2D6}}$ value was 25.4 $\mu\text{L}/\text{min}/\text{pmol}$ and the $CL_{\text{int,CYP2C19}}$ value was 1.84 $\mu\text{L}/\text{min}/\text{pmol}$. Renal clearance was calculated by dividing the total amount of atomoxetine excreted in urine up to infinity by the atomoxetine AUC in CYP2D6 EM subjects following administration of radiolabeled atomoxetine to healthy subjects (Sauer et al., 2003). The inhibition constants of atomoxetine for CYP2D6 and CYP3A4 were collected from the literature (Sauer et al., 2004). All of the PK parameters used for model development are summarized in Table 1 and the final atomoxetine drug model is included in the Supplemental Material. The atomoxetine volume of distribution was predicted using a full PBPK model in Simcyp. Out of the two methods available, method 2 described originally by Rodgers and Rowland (2006) was selected for prediction of the tissue-to-plasma partition coefficients (K_p values) for individual tissues. The predicted volume of distribution

TABLE 1

Physicochemical and PK parameters of atomoxetine used in the PBPK model development

Parameter	Value	Reference
Physicochemical		
Molecular Weight (g/mol)	255.36	Stattera NDA
Log P	3.9	NDA
p <i>K_a</i>	9.8	NDA
<i>f_u</i>	0.025	NDA
B/P	0.623	Simcyp prediction toolbox
Absorption		
<i>F_a</i>	0.96	
<i>k_a</i> (1/h)	1.2	Optimized
<i>Q_{gut}</i> (1/h)	11.9	Simcyp prediction toolbox
Distribution		
<i>V_{ss}</i> (l/kg)	0.74	Simcyp prediction full PBPK method 2
Metabolism/elimination		
<i>CL_{i.v.}</i> (l/h)	16.3	NDA
<i>CL_r</i> (l/h)	0.185	Sauer et al. (2003)
<i>f_{m,CYP2D6}</i>	0.876	
Recombinant <i>CL_{int}</i> ($\mu\text{L}/\text{min}/\text{pmol}$)		
CYP2D6	25.4	
CYP2C19	1.84	
P450 enzyme abundance (pmol/mg microsomal protein)		
CYP2D6 EM	8 (61) ^a	Simcyp default value
CYP2C19 EM	14 (40) ^a	Optimized
Interaction		
CYP2D6 <i>K_i</i> (μM)	34.3	Sauer et al. (2004)
CYP3A4 <i>K_i</i> (μM)	3.6	Sauer et al. (2004)

B/P, blood-to-plasma ratio; *CL_{i.v.}*, clearance after intravenous administration; *CL_r*, renal clearance; *F_a*, fraction absorbed; *f_m*, fraction metabolized; *f_u*, fraction unbound in plasma; *k_a*, absorption rate constant; *K_i*, inhibition constant; Log P, log octanol:water partition coefficient; *Q_{gut}*, drug absorption flow to intestine; *V_{ss}*, volume of distribution at steady state.

^aMean value (coefficient of variance).

at steady state (*V_{ss}*) value was 0.74 l/kg, compared with the observed atomoxetine *V_{ss}* value of 1.02–1.09 l/kg (Center for Drug Evaluation and Research, 2002) following intravenous dosing in healthy subjects.

Simulation of Atomoxetine Disposition and Validation of the Atomoxetine PBPK Model. All simulations were performed using the Simcyp population-based simulator and a full PBPK model. Virtual Simcyp library populations of healthy volunteers, Japanese and Chinese, pediatric population, and organ impairment patients were used. For each simulation, a random seed subject selection was implemented except for DDIs where simulations were conducted as fixed seed to account for the crossover trials. For each simulation, a trial of 100 virtual subjects was used in which the gender and age range of the virtual population matched the reported clinical study data (Supplemental Table 1). If not reported, the gender distribution was set as 1:1 and the age range was set as 18–55. In addition, all simulations only included Caucasian subjects except for specific simulations of other ethnicities since most of the subjects in all studies were Caucasians (Supplemental Table 1) and limited mixed population models are available. The seven PK studies (a training set) listed in Table 2 were used for CYP2D6 *f_m* calculation and model development. After the model was developed, it was validated using 14 trials, which included both CYP2D6 EM and PM populations, single and multiple dosing regimens, and FDA approved and off-label dosing regimens that were not used in model development.

Simulation of Atomoxetine PK in Different Ethnic Groups. To evaluate the applicability of the validated atomoxetine model to PK prediction in different ethnic groups, atomoxetine disposition was simulated in Chinese and Japanese populations using the Simcyp library Chinese, Japanese, and Caucasian populations with and without adjustment for atomoxetine *CL_{int}* based on the activity of CYP2D6*10/*10, a genotype common in Asian populations. First, the Simcyp library Chinese and Japanese population models were directly applied to the validated atomoxetine substrate model. For each population, the phenotype was set as either CYP2D6 EM or intermediate metabolizer (IM) to simulate the CYP2D6 EM and CYP2D6*10/*10 genotyped populations, respectively. Second, the Simcyp library Caucasian population model was used to simulate the Asian population studies to examine the appropriateness of different CYP

TABLE 2
Model development PK data set

The clinical studies used as the training set and the simulated and observed AUC and $C_{\max}$ values for these studies are shown for the EM and PM populations and for the oral and intravenous routes of administration. All trial names are from the NDA file (Center for Drug Evaluation and Research, 2002).

Trial	Dose	AUC			$C_{\max}$		
		Mean Observed	Acceptance Range	Mean Simulated	Mean Observed	Acceptance Range	Mean Simulated
	mg	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	ng/ml	ng/ml	ng/ml
EM population (oral dosing)							
B4L-LC-HFBJ	10 ^a	0.51	0.30–0.88	0.49	85	64–112	79
B4L-LC-HFBJ	90 ^a	5.47	3.17–9.41	4.35	813	618–1073	715
B4Z-LC-LYAE	30 ^b	1.22	0.71–2.10	1.44	320	243–422	278
PM population (oral dosing)							
B4L-LC-HFBJ	10 ^a	4.21	3.20–5.50	5.21	171	127–231	207
B4L-LC-HFBJ	90 ^a	36.7	27.9–48.1	39.7	1518	1123–2049	1237
B4Z-LC-LYAE	30 ^b	11.9	9.04–15.6	12.4	1264	935–1706	1087
EM population (i.v. dosing)							
B4Z-LC-LYAM	20 ^a	1.37	0.77–2.42	1.72	663	504–875	628

^aOnce a day dosing regimen.

^bTwice a day dosing regimen.

enzyme levels in different population models. Third, a reduced atomoxetine CL_{int} value was incorporated in the drug file based on in vitro data of atomoxetine CL_{int} by the CYP2D6*10 enzyme being only 8.6% of the CL_{int} by the CYP2D6*1 enzyme (Shen et al., 2007). To predict the CL_{int} of atomoxetine in the CYP2D6*10/*10 genotyped population, the existing PK data in the CYP2D6 EM phenotype population with mixed genotypes was analyzed based on the CYP2D6 activity score system (Gaedigk et al., 2008) and the known allele frequencies for CYP2D6 genotypes in different populations (Gaedigk et al., 2017). CYP2D6 activity scores were assigned as described for CYP2D6 alleles and their corresponding activity scores (Gaedigk et al., 2008). For example, CYP2D6*3 and CYP2D6*4 were assigned activity score 0, CYP2D6*9 and CYP2D6*10 were assigned activity score 0.5, and CYP2D6*1 and CYP2D6*2 were assigned activity score 1, and the activity scores for each allele were added to obtain the overall activity score for each subject. Of the Caucasian population, 89.3% is phenotypically CYP2D6 EM, which includes CYP2D6 activity scores 1, 1.5, and 2 (Gaedigk et al., 2017). The activity score distribution within only the CYP2D6 EM Caucasian population is 33.5% for activity score 1, 16.6% for activity score 1.5, and 50% for activity score 2. As a result, the expected mean in vivo atomoxetine clearance in the Caucasian population with this distribution of activity scores is expected to be 79% of that in the population with 100% activity score 2 (calculated based on net EM activity being $33.5\% \times 1 + 16.6\% \times 1.5 + 50\% \times 2$ and solving from this the value for activity score 1). Using this calculation the CL_{int} value in a population with 100% activity score 2 (CYP2D6*1) is $32.0 \mu\text{L}/\text{min}/\text{pmol}$ and the CL_{int} value of atomoxetine in CYP2D6*10/*10 individuals is calculated to be $2.77 \mu\text{L}/\text{min}/\text{pmol}$ ($0.086 \times 32 \mu\text{L}/\text{min}/\text{pmol}$). This calculated CL_{int} value in CYP2D6*10/*10 subjects was incorporated into the atomoxetine substrate model when simulating atomoxetine disposition in CYP2D6*10/*10 subjects, and the simulations were repeated using the Simcyp library Chinese, Japanese, and Caucasian EM population models.

Simulation of Atomoxetine PK in DDI Studies. To evaluate the applicability of the validated atomoxetine model to predict DDIs, DDI studies that matched those conducted with atomoxetine were simulated. Four DDI studies were identified, providing atomoxetine PK parameters when administered alone or with known CYP2D6 inhibitors (fluoxetine and paroxetine) or probe substrates midazolam (CYP3A) and desipramine (CYP2D6). For these simulations, previously published fluoxetine, paroxetine, and midazolam models (Ke et al., 2013; Sager et al., 2014) and the Simcyp library desipramine model were used. The performance of these models was evaluated using drug-specific acceptance criteria calculated using the same method as described for atomoxetine and existing PK data for paroxetine (Calvo et al., 2004; Schoedel et al., 2012), midazolam (Eap et al., 2004; Kharasch et al., 2004), and desipramine (Steiner and Spina, 1987; Spina et al., 1993, 1996, 1997) in healthy subjects. The calculation and the acceptance criteria are summarized in Supplemental Tables 5 and 6. The calculated AUC acceptance criterion fold ranges for paroxetine, midazolam, and desipramine were 0.59–1.69, 0.6–1.66, and 0.54–1.85, respectively. The simulated AUC values of all coadministered drugs were within their acceptance

criteria, and hence all coadministered drug models were considered acceptable. Finally, the atomoxetine disposition was simulated in the presence and absence of the coadministered drugs, and the AUC and $C_{\max}$ ratios were computed.

Simulation of Atomoxetine PK in Special Populations. To evaluate whether the atomoxetine model validated in healthy adults could be used to predict atomoxetine PK in pediatric population, ESRD patients, and HI patients, atomoxetine disposition in these populations was simulated using the existing Simcyp library special population models. Based on the evaluation of atomoxetine PK variability in these populations, the model performance was assessed using the same criterion as presented for the healthy adult population. For pediatrics simulation, the Simcyp library pediatric population model was used with corresponding CYP2D6 genotype information provided in each study. For HI simulation, the Simcyp Child-Pugh B and Child-Pugh C population models with CYP2D6 EM were used according to the reported trials (Center for Drug Evaluation and Research, 2002; Chalon et al., 2003). For ESRD, the Simcyp eGFR <30 L/h population was used according to the data in the atomoxetine NDA. Since the ESRD clinical study did not specify the CYP2D6 genotypes of the subjects, the CYP2D6 genotype distribution was set as the Simcyp default, i.e., 85% EM, 8% PM, and 7% ultrarapid metabolizer in the ESRD population model. In addition, due to the limited number of subjects ($N = 6$) in the ESRD study, 50 trials of six subjects were simulated using random seed selection to capture possible interstudy variability due to small sample size and variable genotype distribution. Dialysis data were not available for the ESRD population and hence were not included in the simulation.

Results

Atomoxetine PBPK Model Development and Validation. The atomoxetine PBPK model (Table 1) was first developed and optimized using a training data set (see the Supplemental Material for compound file and representative model output). The training set included both intravenous and oral dosing regimens and CYP2D6 EM and PM populations (Fig. 1). All of the simulated AUC and $C_{\max}$ values of the training set studies met the predefined drug-specific model acceptance criteria for AUC and $C_{\max}$ (Table 2), confirming the model parameters such as absorption rate constant, bioavailability, clearance, and V_{ss} . The model was then validated using a separate test data set of 14 human PK studies including both CYP2D6 EM and PM populations, single and multiple dosing regimens, and FDA approved and off-label dosing regimens (Table 3). Similar to the training data set, all of the simulated AUC and $C_{\max}$ values for the validation studies were within the acceptance criteria (Table 3). Therefore, the model was considered validated for healthy adult Caucasian subjects with CYP2D6 EM and PM genotypes.

TABLE 3
Model validation PK data set

The clinical studies used as the validation set and the simulated and observed mean AUC and $C_{\max}$ values for these studies are shown for the EM and PM populations and for the approved and off-label dosing regimens. All trial names are from the NDA file (Center for Drug Evaluation and Research, 2002).

Trial	Dose	AUC			$C_{\max}$		
		Mean Observed	Acceptance Range	Mean Simulated	Mean Observed	Acceptance Range	Mean Simulated
	mg	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	ng/ml	ng/ml	ng/ml
EM population ^a							
B4Z-LC-LYAM	40 ^b	1.80	1.04–3.10	1.82	326	248–430	341
B4Z-LC-LYAL	40 ^b	2.11	1.22–3.63	1.92	333	253–440	318
B4Z-LC-LYAZ	60 ^b	3.02	1.75–5.19	2.8	529	402–698	477
B4L-LC-HFBH	20 ^c	1.08	0.63–1.86	1.16	160	121–211	200
B4Z-LC-LYAE	45 ^c	1.97	1.14–3.39	2.16	490	372–647	416
PM population ^a							
B4Z-LC-LYAK	40 ^b	14.5	11.0–19.0	15.9	564	417–761	568
B4L-LC-HFBH	20 ^c	8.44	6.41–11.1	9.51	915	677–1235	917
B4Z-LC-LYAE	45 ^c	18.0	13.7–23.6	18.6	1868	1382–2522	1631
EM population ^d							
B4L-LC-HFBJ	120 ^b	7.42	4.16–13.1	5.64	1053	800–1390	1000
B4Z-LC-LYAE	60 ^c	2.67	1.50–4.73	3.03	646	491–852	593
B4Z-LC-LYAE	75 ^c	3.70	2.07–6.55	3.81	821	624–1084	723
PM population ^d							
B4L-LC-HFBJ	120 ^a	51.6	38.2–69.7	48.9	2233	1675–2992	1829
B4Z-LC-LYAE	60 ^b	26.7	19.8–36.0	26.1	2919	2189–3911	2591
B4Z-LC-LYAE	75 ^b	37.4	27.7–50.5	33.2	3999	2999–5359	3288

^aFDA approved dosing regimen.

^bOnce a day dosing regimen.

^cTwice a day dosing regimen.

^dOff-label dosing regimen.

Simulation of Atomoxetine PK in Different Ethnicities. To test whether the atomoxetine model validated in Caucasians could be used to predict atomoxetine disposition in Chinese and Japanese populations, atomoxetine disposition was simulated using the Simcyp built-in Chinese and Japanese population models without any modifications (Table 4). Since the reported studies showed PK data separately for CYP2D6 EM and CYP2D6*10/*10 genotyped subjects, atomoxetine disposition was separately simulated using the Chinese and Japanese CYP2D6 EM and IM populations. Overall, when using these populations the majority of the simulated PK parameters did not pass the model acceptance criteria (Table 4). For CYP2D6 EM subjects, only 38% of the simulated PK parameters met the study specific acceptance criteria and even these simulations resulted in predictions close to the upper limit of the acceptance range. For CYP2D6*10/*10 genotyped subjects (considered as Japanese and Chinese IM subjects), 63% of the simulated PK parameters met the acceptance criteria. Therefore, compared with the 100% simulation success rate in the 14 validation studies, the predictions of atomoxetine disposition in Asian populations using Simcyp Asian population models was unacceptable. Of note, the Chinese and Japanese population models have presumptively lower CYP2D6 expression than the Caucasian population. This may result in simulation failure if Caucasian and Asian subjects of similar genotype (same activity score) actually have similar CYP2D6 protein expression levels. To test this, atomoxetine disposition in Japanese and Chinese populations was simulated using the Caucasian population model. Doing so, 100% atomoxetine PK parameters in Chinese and Japanese EM subjects were acceptably predicted. In addition, the atomoxetine specific CL_{int} changes in CYP2D6*10/*10 subjects were incorporated into the model based on published in vitro data, to test whether in vitro pharmacogenetic data could be used to reliably predict in vivo disposition in this genotype group. Doing so, the Chinese and Japanese population models poorly predicted atomoxetine PK parameters in Chinese and Japanese CYP2D6*10/*10 subjects with 0% success rate. On the other hand, after applying the CL_{int} changes the Caucasian population model

predicted the disposition of atomoxetine in Chinese and Japanese CYP2D6*10/*10 subjects with 75% success rate.

Simulation of Atomoxetine PK in DDI Studies. To evaluate the applicability of the validated model in predicting atomoxetine disposition in DDI scenarios, atomoxetine disposition was simulated when coadministered with the CYP2D6 inhibitors fluoxetine and paroxetine and the probe drugs desipramine and midazolam (Table 5). The PBPK models for all of the coadministered drugs resulted in simulated AUC and $C_{\max}$ values within their acceptance criteria (Supplemental Table 7). Overall, for a total of 12 simulated DDI trials, the atomoxetine AUC value was predicted acceptably in 11 (92%) trials and the $C_{\max}$ value was predicted acceptably in eight (67%) trials (Table 5). The only study in which the atomoxetine AUC value was not predicted within the acceptance criteria was a study in which 40 mg atomoxetine was coadministered with desipramine (Table 5). In the same study, the AUC value following 60 mg dosing of atomoxetine was accurately predicted. In the observed data, there was no dose-proportional increase in the atomoxetine AUC value between the 40 mg ($n = 6$) and 60 mg ($n = 15$) dose groups despite atomoxetine having linear kinetics. Based on this discrepancy in the experimental data, possibly due to the small sample size ($N = 6$) in the 40 mg dose group, the 92% performance rate of the model was considered acceptable.

The effect of CYP2D6 inhibition by paroxetine on the atomoxetine AUC and $C_{\max}$ values was well predicted. In the two paroxetine DDI studies, 100% of the atomoxetine AUC and $C_{\max}$ values met the acceptance criteria (Table 6). Furthermore, in the two studies the atomoxetine AUC values were reported to increase 7.0-fold (Center for Drug Evaluation and Research, 2002) and 5.6-fold (Todor et al., 2015), respectively, and the corresponding predicted increases were 6.7-fold and 5.9-fold. Similarly, the atomoxetine $C_{\max}$ values were reported to increase 3.8-fold (Center for Drug Evaluation and Research, 2002) and 1.7-fold (Todor et al., 2015), while 3.9- and 1.5-fold increases in the $C_{\max}$ values were predicted in the two studies, respectively. For the paroxetine DDI study with available plasma-concentration time data points, all of the observed data points were within the 95% confidence interval (CI) of the simulations (Fig. 2). These

TABLE 4
Simulated and observed atomoxetine PK in different ethnic populations

Atomoxetine disposition in Chinese and Japanese subjects was simulated using the different models in Simcyp as described in Materials and Methods. Bold values are outside the acceptance range.

CYP2D6 Status	Observed Value (Acceptance Range) ^a	Simulated Asian Population ^a	Simulated Caucasian Population ^a
AUC ($\mu\text{g}\cdot\text{hr}/\text{mL}$)			
Chinese study 40 mg single dose ^b			
EM	2.24 (1.28–3.97)	3.53	1.95
CYP2D6*10/*10 (IM)	4.96 (2.82–8.78)	7.62	
CYP2D6*10/*10 adjusted ^b		18.2	8.10
Chinese study 80 mg every day for 7 days			
EM	4.43 (2.52–7.84)	7.06	3.36
CYP2D6*10/*10 (IM)	9.69 (5.53–17.2)	16.3	
CYP2D6*10/*10 adjusted ^b		35.8	16.3
Japanese study 10 mg single dose ^c			
EM	0.44 (0.25–0.78)	0.93	0.38
CYP2D6*10/*10 (IM)	0.71 (0.41–1.26)	2.13	
CYP2D6*10/*10 adjusted ^b		6.80	2.03
Japanese study 120 mg single dose ^c			
EM	5.26 (3.00–9.31)	11.2	4.65
CYP2D6*10/*10 (IM)	9.8 (5.59–17.3)	25.5	
CYP2D6*10/*10 adjusted ^b		81.7	24.0
C_{max} (ng/mL)			
Chinese study 40 mg single dose			
EM	360 (247–472)	480	343
CYP2D6*10/*10 (IM)	530 (403–694)	600	
CYP2D6*10/*10 adjusted ^b		709	543
Chinese study 80 mg every day for 7 days			
EM	815 (619–1068)	1079	651
CYP2D6*10/*10 (IM)	1199 (911–1571)	1499	
CYP2D6*10/*10 adjusted ^b		2319	1379
Japanese study 10 mg single dose ^d			
EM	102 (77.2–133)	124	86
CYP2D6*10/*10 (IM)	125 (95.1–164)	160	
CYP2D6*10/*10 adjusted ^b		181	136
Japanese study 120 mg single dose ^d			
EM	978 (743–1281)	1490	860
CYP2D6*10/*10 (IM)	1271 (966–1665)	1880	
CYP2D6*10/*10 adjusted ^b		2178	1630

IM, intermediate metabolizer.

^aGeometric mean.

^bThe CL_{int} value for atomoxetine was adjusted based on in vitro data as discussed in *Materials and Methods*.

^cTrial B4Z-LE-LYAN; the AUC values are given ($\mu\text{g}\cdot\text{h}/\text{mL}$) (Center for Drug Evaluation and Research, 2002).

^dTrial B4Z-LE-LYAN; the C_{max} values are given (ng/mL) (Center for Drug Evaluation and Research, 2002).

results demonstrate excellent agreement between the observed and simulated DDIs. However, in the presence of fluoxetine, the atomoxetine C_{max} values were underpredicted despite the AUC values being within the acceptance criteria. Since the plasma concentration-time curves for this study were not available, the quality of the experimental data for C_{max} determination could not be assessed. The fold change in the atomoxetine AUC value in the presence of fluoxetine could not be evaluated since the data from the control session in this study were not available. As expected, there was no change in the atomoxetine AUC or C_{max} value after coadministration of midazolam or desipramine (both administered as a single dose) with atomoxetine (Table 5). Importantly, the PBPK modeling also accurately predicted midazolam and desipramine disposition in the presence and absence of coadministered atomoxetine and the lack of a DDI in these scenarios (Supplemental Table 7).

Simulation of Atomoxetine PK in Special Populations. To evaluate the applicability of the validated model in predicting atomoxetine disposition in special populations, atomoxetine disposition was simulated in pediatric subjects, ESRD, and HI patients. When atomoxetine disposition was simulated in the pediatric population, all simulated AUC and C_{max} values for the four trials were within the acceptance criteria (Table 6). Based on these data, the validated model could be successfully applied to predict atomoxetine disposition in both EM and PM pediatric

populations. The observed plasma concentration-time curves for the pediatric trials were all within the 95% CI of the predicted mean (Fig. 3).

The predicted atomoxetine AUC value in ESRD was 2-fold greater than that in the matching healthy population (Table 6). This magnitude of increase in the atomoxetine AUC value (the ratio of the AUC in ESRD over healthy) is in agreement with the reported data of the AUC change in ESRD (Center for Drug Evaluation and Research, 2002) (Table 6). However, neither the overall simulated AUC nor the C_{max} met the acceptance criteria in either healthy subjects or in the ESRD patients. This could potentially be due to the small sample size ($n = 6$) of the ESRD study and the variability of the data in this population. When the 50 simulated trials of ESRD patients were assessed, only 20% of these met the acceptance criteria of the AUC (Fig. 4) and none of them met the acceptance criteria of C_{max}. It is also noteworthy that the observed AUC in the healthy subject group in the ESRD study would not have met the acceptance criteria for the atomoxetine AUC in the healthy subject studies with the same dosage (20 mg) and this AUC is not within the observed AUCs in the healthy population.

When atomoxetine disposition was simulated in the population with moderate and severe HI in comparison with healthy controls, a 4- to 10-fold increase in the atomoxetine AUC value was predicted in HI compared with the control (Table 6). This predicted effect of HI greatly

TABLE 5
Simulated and observed atomoxetine PK in DDI studies

The observed and predicted mean atomoxetine AUC and C_{max} values together with the acceptance criteria for each study are listed. For the DDI studies both study sessions (control and coadministration) were simulated and are reported. All studies were conducted in EM populations except ATM + MDZ. Bold values are outside the acceptance range.

Trial	Dose	AUC			C_{max}		
		Mean Observed	Acceptance Range	Mean Simulated	Mean Observed	Acceptance range	Mean Simulated
	mg	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	ng/ml	ng/ml	ng/ml
ATM + FLX ^a	10	2.82	1.64–4.85	2.02	328	249–433	242
	45	14.4	8.35–24.8	9.09	1686	1281–2226	1110
ATM ^a	20	0.85	0.491–1.46	0.95	184	139–243	165
ATM + PRX ^a	20	5.97	3.46–10.3	6.39	690	524–911	643
ATM ^b	25	1.15	0.65–2.03	1.13	221	168–290	204
ATM + PRX ^b	25	6.45	3.64–11.4	6.64	373	283–489	304
ATM ^a	40	3.18	1.84–5.47	1.94	552	420–729	351
	60	2.69	1.56–4.63	2.92	591	449–780	527
ATM + DES ^a	40	3.47	2.01–5.97	1.94	557	423–735	351
	60	3.01	1.75–5.18	2.92	647	492–854	527
ATM + MDZ ^{a,c}	60	23.4	17.8–30.7	24.7	2610	1931–3524	2454
	60	24.3	18.5–31.8	27.1	2694	1996–3637	2663

ATM, atomoxetine; DES, desipramine; FLX, fluoxetine; MDZ, midazolam; PRX, paroxetine.

^aData from the atomoxetine NDA submission (Center for Drug Evaluation and Research, 2002).

^bData from Todor et al. (2015).

^cData from CYP2D6 PM subjects.

exceeded the observed 1- to 2.5-fold increase in the atomoxetine AUC value in HI. While the model predicted atomoxetine disposition in healthy subjects within the acceptance criteria, both the AUC and C_{max} values were significantly overpredicted in the HI populations and these simulations did not meet the model acceptance criteria.

Discussion

The goal of this study was to evaluate whether a drug PBPK model validated against a comprehensive in vivo PK data set in healthy

volunteers with different genotypes could be used with previously developed population models to simulate drug disposition in specific patient groups. Atomoxetine, a well-characterized CYP2D6 probe was used as the model drug. A full PBPK model was developed and validated for atomoxetine, and this model can be used in the future to simulate atomoxetine disposition in healthy volunteers. This study is the first of its kind to cross-evaluate population models for a probe drug with a validated drug model. Overall, the findings were consistent with previous reports emphasizing low confidence in population models for HI, ESRD, and different ethnicities, but confirm high confidence in

TABLE 6
Simulated and observed atomoxetine PK in special populations

The observed and predicted mean atomoxetine AUC and C_{max} values together with the acceptance criteria for each study are listed. The observed and simulated data are shown for genotyped pediatric populations, patients with ESRD, and healthy controls in the same study and for HI patients (Child-Pugh B and Child-Pugh C) and controls in the same study. For the ESRD and HI studies, the ratios of the PK values in disease versus healthy was calculated for observed and simulated data and are also reported. Bold values are outside the acceptance range.

Population	AUC			C_{max}		
	Mean Observed	Acceptance Range	Mean Simulated	Mean Observed	Acceptance Range	Mean Simulated
	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	$\mu\text{g}\cdot\text{h}/\text{ml}$	ng/ml	ng/ml	ng/ml
Pediatric population ^a						
EM ^b	0.65	0.364–1.14	1.07	144	109–189	167
EM 1 ^c	0.89	0.505–1.58	1.17	179	136–234	225
EM 2 ^d	1.23	0.692–2.17	1.17	255	194–334	225
PM ^e	12.7	9.35–17.1	12.4	638	479–836	500
ESRD patient population ^f						
Healthy	0.50	0.28–0.88	1.47	92.3	70.1–121	184
ESRD	1.00	0.57–1.77	2.98	105	80.1–138	230
Ratio of E-H	2.00		2.03	1.14		1.25
HI patient population ^g						
Healthy subjects	0.69	0.394–1.22	0.97	0.142	0.081–0.251	0.17
CP-B	1.16	0.661–2.05	4.63	0.115	0.066–0.204	0.32
CP-C	2.54	1.45–4.50	10.4	0.125	0.071–0.221	0.41
Ratio of CP-B/H	1.68		4.77	0.81		1.88
Ratio of CP-C/H	3.67		10.7	0.88		2.41

CP-B, Child-Pugh B; CP-C, Child-Pugh C; H, healthy.

^aData from Center for Drug Evaluation and Research (2002) and Brown et al. (2016).

^bData from atomoxetine NDA submission file with CYP2D6 EM phenotype population (Center for Drug Evaluation and Research, 2002).

^cData from subjects with CYP2D6 activity score of 1 (data from Brown et al. (2016)).

^dData from subjects with CYP2D6 activity score of 2 (data from Brown et al. (2016)).

^eData from CYP2D6 PM subjects (data from Brown et al. (2016)).

^fTrial B4Z-LC-HFBM (Center for Drug Evaluation and Research, 2002).

^gTrial B4Z-LC-HFBN (Center for Drug Evaluation and Research, 2002).

Fig. 2. Simulated and observed plasma concentration time curves for atomoxetine in the presence of placebo (A) and paroxetine (B). The red dots indicate the observed data with its S.D., the black line shows the mean values of the simulated data, and the blue dashed lines show the 95% CIs of the simulated data.

PBPK modeling in healthy volunteers and DDI studies (Jones et al., 2015; Wagner et al., 2015). Importantly, due to the rigorous model development/validation/extrapolation workflow employed, the findings of this study allow differentiating potential causes of low confidence in specific population simulations including questions of clinical data quality and uncertainties in population models.

It has been suggested that small clinical studies and high intrinsic variability in PK contribute to poor model performance (Abduljalil et al., 2014). To address this issue, this study used a statistical model acceptance criterion (Abduljalil et al., 2014) that is calculated specifically for the drug of interest based on the observed PK variability of that drug. This criterion is based on the 99.998% CI of all observed clinical data, and hence it is expected that nearly all simulations will be within this criterion regardless of the study size or data variability since only 0.002% of clinical data should be outside of the criterion range. For atomoxetine, due to the relatively low variability in its disposition, the calculated 99.998% CI was more stringent than the commonly used 2-fold criterion. The acceptance range was 0.56- to 1.77-fold for the EM population and 0.74- to 1.35-fold for the PM population. This is in agreement with previous studies stating that the 2-fold criterion may be scientifically too lenient to assess model quality for some drugs (Abduljalil et al., 2014). The statistical criterion for model evaluation also shows that due to the variability in the atomoxetine AUC in the healthy population, the model is not expected to simulate the atomoxetine

AUC within a criterion analogous to bioequivalence (1.25-fold) (Guest et al., 2011) in 9.7% of healthy volunteer studies in EMs (calculated based on the critical value of 1.658 for 1.25-fold error from eqs. 1–3). Indeed, 25% of the AUC values in the verification and validation studies in healthy volunteers with the EM phenotype were not simulated within the 1.25-fold criterion (Supplemental Table 8). In contrast, as predicted from the lower variability in the PM populations, the atomoxetine AUC value was predicted within 1.25-fold in all of the CYP2D6 PM studies. Taken together, this analysis shows that the commonly used goal of fit-for-purpose for PBPK model performance should be considered within the context of the intrinsic variability of the drug of interest. Essentially, the criterion used here can be applied to establish confidence regarding what fraction of clinical trials are expected to be predicted within the fit-for-purpose n -fold criteria and to determine how many clinical studies should be analyzed to establish confidence in the drug model.

The data shown here clearly identify population models as the primary reason for poor PBPK model extrapolation regardless of the model acceptance criteria used. The role of population models in contributing to poor simulation performance is clearly shown in the simulation of Asian populations using the population models for Caucasians, Japanese, and Chinese. Atomoxetine disposition in Japanese and Chinese EM subjects was best predicted using the Caucasian EM population model instead of the Chinese or Japanese population models. The CYP2D6*10 allele has been shown to be much more prevalent in Asian populations than in

Fig. 3. Simulated and observed plasma concentration time curves for atomoxetine for the pediatric trials reported in the literature. (A) Simulation of the plasma-concentration time curve from the publicly available NDA file. (B–D) Plasma-concentration time curves in genotyped pediatric subjects with an activity score of 1 (B), activity score of 2 (C), or a PM genotype (D) as reported for observed data in Brown et al. (2016). The red dots indicate the observed data with its S.D., the black line shows the mean values of the simulated data, and the blue dashed lines show the 95% CIs of the simulated data.

Fig. 4. Simulated AUC and $C_{\max}$ values for the 50 individual trials in the healthy patients (A and B) and in ESRD patients (C and D) in comparison with the observed study data and its S.D. None of the subjects in this study were genotyped.

Caucasians (Kitada, 2003), resulting in apparent lower population CYP2D6 activity. In agreement with this phenotype, the Chinese and Japanese population models in Simcyp have lower CYP2D6 expression levels (4 and 4.5 pmol/mg) than the Caucasian population (8 pmol/mg). The low CYP2D6 expression levels in the Asian population models explain the simulation failures observed with Japanese and Chinese population studies, and the data obtained here strongly suggest CYP2D6 protein expression levels are similar between Asian and Caucasian populations. If the populations are genotyped for CYP2D6, the Caucasian population model is superior to the Asian population models. Since atomoxetine is a well-characterized CYP2D6 probe, it is likely that these results can be extrapolated to other CYP2D6 substrates as well. The fact that the Caucasian model with the CL_{int} value adjusted based on the CYP2D6*10/*10 genotype overpredicted 25% of the observed atomoxetine PK parameters (AUC and $C_{\max}$) may be due to poor extrapolation of in vitro to in vivo enzyme activity. This interpretation is supported by the fact that while in vitro CYP2D6*10 had only 8% of the activity of CYP2D6*1 (Shen et al., 2007), the in vivo activity score of CYP2D6*10 is 0.5 (Gaedigk et al., 2008), suggesting only 50% difference in the in vivo activity between CYP2D6*10 and CYP2D6*1.

The results of this study support the consensus that PBPK models can be used to predict drug disposition in pediatric populations above 2 years of age (Jones et al., 2015; Wagner et al., 2015). The model accurately predicted atomoxetine disposition in pediatric populations with defined genotypes, suggesting that the disposition of CYP2D6 substrates can be extrapolated using full PBPK models from adult studies to pediatrics even in different genotype groups. In contrast, the validated atomoxetine model could not reliably predict atomoxetine disposition in ESRD or HI using the existing RI and HI population models. It is highly unlikely that this failure is due to the small sample size ($n = 6, 6,$ and 4 in the ESRD, Child-Pugh B, and Child-Pugh C trials, respectively) or high variability of atomoxetine disposition in these studies. The AUC acceptance criteria calculated based on the observed variability and sample size in the organ impairment studies were 0.34- to 2.92-fold for the ESRD trial, 0.54- to 1.86-fold for the Child-Pugh B trial, and 0.29- to 3.43-fold for the

Child-Pugh C trial, and the simulated AUC values did not meet these acceptance criteria. Similarly, all of these simulations also failed when using the common 2-fold criterion (Supplemental Table 8). Atomoxetine is almost entirely cleared via hepatic metabolism and as such the population model parameters that result in the simulated 2-fold increase in the atomoxetine AUC value in the ESRD population are not clear. The current label of atomoxetine states that RI does not affect atomoxetine disposition, presumably because the body weight normalized clearance of atomoxetine is not different in ESRD patients and controls despite the 2-fold change in the AUC of atomoxetine. This lack of change in clearance of atomoxetine in the ESRD group was not captured here, suggesting that the confidence in the ESRD population model for at least CYP2D6 substrates is low. The poor performance of the ESRD model was unexpected since previous studies have successfully (within 1.5-fold) simulated bisoprolol disposition in RI populations using the full PBPK model (Li et al., 2012) and orteronel disposition in RI populations using the minimal PBPK model (Lu et al., 2014). However, no comprehensive model validation across healthy volunteers and different special populations was presented in these studies. Since both of these drugs are mainly renally cleared, it is possible that the simulation failure here is specific to metabolically cleared drugs. In addition, differences between the full PBPK and minimal PBPK model populations may contribute to the simulation failure, and further studies with a broader range of drugs are needed to evaluate these possibilities. It is known that RI can affect the activity of drug metabolizing enzymes in the liver and the clearance and distribution characteristics of mainly metabolically cleared drugs (Touchette and Slaughter, 1991; Zhao et al., 2012). It has also been suggested that RI affects CYP2D6 cleared drugs more than some of the other CYP enzymes (Touchette and Slaughter, 1991; Zhao et al., 2012). The results here suggest that this hepatorenal coupling in renal disease is not sufficiently well characterized to allow reliable simulations of CYP2D6 substrates in RI, and the effect of RI on hepatic clearance may be overestimated.

For the HI studies, the overprediction in the AUC value is likely because of overestimation in the magnitude of the decrease in CYP2D6 expression and liver size in the Simcyp HI population model. In the Simcyp library Child-Pugh B and Child-Pugh C population models,

CYP2D6 expression is decreased by 67.5% and 89.5%, respectively, and the liver volume is similarly decreased by 29% and 39.4%, respectively. The data collected here suggest that this is an overestimation of the decrease in CYP2D6 expression/activity in the HI population. Using the validated atomoxetine model, the decrease in CYP2D6 expression in the HI population can be estimated based on the observed 1.7- to 3.7-fold increase in the atomoxetine AUC value in moderate and severe HI. If the change in liver volume is kept as defined in the HI population model, no change occurs in CYP2D6 expression in moderate HI patients, and a decrease of 25% in CYP2D6 expression in severe HI patients is estimated. In contrast, if there is no change in liver volume, the CYP2D6 expression level is predicted to decrease 32% and 89% in moderate and severe HI patients, respectively. A larger number of CYP2D6 substrates needs to be studied via rigorous simulation approaches to determine whether the predicted percentage change in CYP2D6 expression is correct in HI. In addition, further studies using CYP2D6 substrates with different plasma protein binding and different effects of HI on plasma protein binding need to be evaluated to delineate altered plasma protein binding in HI from altered CYP activity in contributing to simulation accuracy. However, taken together, these data demonstrate a lack of adequate knowledge and confidence in the physiologic changes and enzyme expression in organ impairment populations to allow PBPK-based predictions.

Authorship Contributions

Participated in research design: Huang, Ragueneau-Majlessi, Isoherranen.

Conducted experiments: Huang, Nakano, Sager.

Performed data analysis: Huang, Isoherranen.

Wrote or contributed to the writing of the manuscript: Huang, Nakano, Sager, Ragueneau-Majlessi, Isoherranen.

References

- Abduljalil K, Cain T, Humphries H, and Rostami-Hodjegan A (2014) Deciding on success criteria for predictability of pharmacokinetic parameters from in vitro studies: an analysis based on in vivo observations. *Drug Metab Dispos* **42**:1478–1484.
- Belle DJ, Ernest CS, Sauer JM, Smith BP, Thomasson HR, and Witcher JW (2002) Effect of potent CYP2D6 inhibition by paroxetine on atomoxetine pharmacokinetics. *J Clin Pharmacol* **42**:1219–1227.
- Brown JT, Abdel-Rahman SM, van Haandel L, Gaedigk A, Lin YS, and Leeder JS (2016) Single dose, CYP2D6 genotype-stratified pharmacokinetic study of atomoxetine in children with ADHD. *Clin Pharmacol Ther* **99**:642–650.
- Calvo G, García-Gea C, Luque A, Morte A, Dal-Ré R, and Barbanj M (2004) Lack of pharmacologic interaction between paroxetine and alprazolam at steady state in healthy volunteers. *J Clin Psychopharmacol* **24**:268–276.
- Center for Drug Evaluation and Research (2002) Application number 21-411. Clinical Pharmacology and biopharmaceutics review(s) https://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-411_Strattera.cfm.
- Center for Drug Evaluation and Research (2012) *Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*, U.S. Food and Drug Administration, Silver Spring, MD.
- Chalon SA, Desager JP, Desante KA, Frye RF, Witcher J, Long AJ, Sauer JM, Golnez JL, Smith BP, Thomasson HR, et al. (2003) Effect of hepatic impairment on the pharmacokinetics of atomoxetine and its metabolites. *Clin Pharmacol Ther* **73**:178–191.
- Committee for Medicinal Products for Human Use, European Medicines Agency (2005) *Guideline on the Evaluation of the Pharmacokinetics of Medicinal Products in Patients with Impaired Hepatic Function*. CPMP/EWP/2339/02, European Medicines Agency, London.
- Choi CI, Bae JW, Lee YJ, Lee HI, Jang CG, and Lee SY (2014) Effects of CYP2C19 genetic polymorphisms on atomoxetine pharmacokinetics. *J Clin Psychopharmacol* **34**:139–142.
- Cui YM, Teng CH, Pan AX, Yuen E, Yeo KP, Zhou Y, Zhao X, Long AJ, Bangs ME, and Wise SD (2007) Atomoxetine pharmacokinetics in healthy Chinese subjects and effect of the CYP2D6*10 allele. *Br J Clin Pharmacol* **64**:445–449.
- Eap CB, Buclin T, Cucchia G, Zullino D, Hustert E, Bleiber G, Golay KP, Aubert AC, Baumann P, Telenti A, et al. (2004) Oral administration of a low dose of midazolam (75 µg) as an in vivo probe for CYP3A activity. *Eur J Clin Pharmacol* **60**:237–246.
- Edginton AN, Schmitt W, and Willmann S (2006) Development and evaluation of a generic physiologically based pharmacokinetic model for children. *Clin Pharmacokinet* **45**:1013–1034.
- Edginton AN and Willmann S (2008) Physiology-based simulations of a pathological condition: prediction of pharmacokinetics in patients with liver cirrhosis. *Clin Pharmacokinet* **47**:743–752.
- Gaedigk A, Sangkuhl K, Whirl-Carrillo M, Klein T, and Leeder JS (2017) Prediction of CYP2D6 phenotype from genotype across world populations. *Genet Med* **19**:69–76.
- Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, and Leeder JS (2008) The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. *Clin Pharmacol Ther* **83**:234–242.
- Guest EJ, Aarons L, Houston JB, Rostami-Hodjegan A, and Galetin A (2011) Critique of the two-fold measure of prediction success for ratios: application for the assessment of drug-drug interactions. *Drug Metab Dispos* **39**:170–173.
- Huang SM, Abernethy DR, Wang Y, Zhao P, and Zineh I (2013) The utility of modeling and simulation in drug development and regulatory review. *J Pharm Sci* **102**:2912–2923.
- Huang SM and Rowland M (2012) The role of physiologically based pharmacokinetic modeling in regulatory review. *Clin Pharmacol Ther* **91**:542–549.
- Ito K, Hallifax D, Obach RS, and Houston JB (2005) Impact of parallel pathways of drug elimination and multiple cytochrome P450 involvement on drug-drug interactions: CYP2D6 paradigm. *Drug Metab Dispos* **33**:837–844.
- Jamei M, Marciniak S, Feng K, Barnett A, Tucker G, and Rostami-Hodjegan A (2009) The Simcyp® population-based ADME simulator. *Expert Opin Drug Metab Toxicol* **5**:211–223.
- Johnson TN, Boussey K, Rowland-Yeo K, Tucker GT, and Rostami-Hodjegan A (2010) A semi-mechanistic model to predict the effects of liver cirrhosis on drug clearance. *Clin Pharmacokinet* **49**:189–206.
- Johnson TN, Rostami-Hodjegan A, and Tucker GT (2006) Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. *Clin Pharmacokinet* **45**:931–956.
- Jones HM, Chen Y, Gibson C, Heimbach T, Parrott N, Peters SA, Snoeys J, Upreti VV, Zheng M, and Hall SD (2015) Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clin Pharmacol Ther* **97**:247–262.
- Jones HM, Gardner IB, and Watson KJ (2009) Modelling and PBPK simulation in drug discovery. *AAPS J* **11**:155–166.
- Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, Isoherranen N, and Unadkat JD (2013) A physiologically based pharmacokinetic model to predict disposition of CYP2D6 and CYP1A2 metabolized drugs in pregnant women. *Drug Metab Dispos* **41**:801–813.
- Kharasch ED, Walker A, Hoffer C, and Sheffels P (2004) Intravenous and oral alfentanil as in vivo probes for hepatic and first-pass cytochrome P450 3A activity: noninvasive assessment by use of pupillary miosis. *Clin Pharmacol Ther* **76**:452–466.
- Kitada M (2003) Genetic polymorphism of cytochrome P450 enzymes in Asian populations: focus on CYP2D6. *Int J Clin Pharmacol Res* **23**:31–35.
- Li GF, Wang K, Chen R, Zhao HR, Yang J, and Zheng QS (2012) Simulation of the pharmacokinetics of bisoprolol in healthy adults and patients with impaired renal function using whole-body physiologically based pharmacokinetic modeling. *Acta Pharmacol Sin* **33**:1359–1371.
- Lu C, Suri A, Shyu WC, and Prakash S (2014) Assessment of cytochrome P450-mediated drug-drug interaction potential of oroteronel and exposure changes in patients with renal impairment using physiologically based pharmacokinetic modeling and simulation. *Biopharm Drug Dispos* **35**:543–552.
- Matsui A, Azuma J, Witcher JW, Long AJ, Sauer JM, Smith BP, DeSante KA, Read HA, Takahashi M, and Nakano M (2012) Pharmacokinetics, safety, and tolerability of atomoxetine and effect of CYP2D6*10/*10 genotype in healthy Japanese men. *J Clin Pharmacol* **52**:388–403.
- Ring BJ, Gillespie JS, Eckstein JA, and Wrighton SA (2002) Identification of the human cytochromes P450 responsible for atomoxetine metabolism. *Drug Metab Dispos* **30**:319–323.
- Rodgers T and Rowland M (2006) Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J Pharm Sci* **95**:1238–1257.
- Rowland M, Peck C, and Tucker G (2011) Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu Rev Pharmacol Toxicol* **51**:45–73.
- Rowland Yeo K, Aarabi M, Jamei M, and Rostami-Hodjegan A (2011) Modeling and predicting drug pharmacokinetics in patients with renal impairment. *Expert Rev Clin Pharmacol* **4**:261–274.
- Sager JE, Lutz JD, Foti RS, Davis C, Kunze KL, and Isoherranen N (2014) Fluoxetine- and norfluoxetine-mediated complex drug-drug interactions: in vitro to in vivo correlation of effects on CYP2D6, CYP2C19, and CYP3A4. *Clin Pharmacol Ther* **95**:653–662.
- Sauer JM, Long AJ, Ring B, Gillespie JS, Sanburn NP, DeSante KA, Petullo D, VandenBranden MR, Jensen CB, Wrighton SA, et al. (2004) Atomoxetine hydrochloride: clinical drug-drug interaction prediction and outcome. *J Pharmacol Exp Ther* **308**:410–418.
- Sauer JM, Ponsler GD, Mattiuz EL, Long AJ, Witcher JW, Thomasson HR, and Desante KA (2003) Disposition and metabolic fate of atomoxetine hydrochloride: the role of CYP2D6 in human disposition and metabolism. *Drug Metab Dispos* **31**:98–107.
- Schoedel KA, Pope LE, and Sellers EM (2012) Randomized open-label drug-drug interaction trial of dextromethorphan/quinidine and paroxetine in healthy volunteers. *Clin Drug Invest* **32**:157–169.
- Shen H, He MM, Liu H, Wrighton SA, Wang L, Guo B, and Li C (2007) Comparative metabolic capabilities and inhibitory profiles of CYP2D6.1, CYP2D6.10, and CYP2D6.17. *Drug Metab Dispos* **35**:1292–1300.
- Spina E, Avenoso A, Campo GM, Caputi AP, and Perucca E (1996) Phenobarbital induces the 2-hydroxylation of desipramine. *Ther Drug Monit* **18**:60–64.
- Spina E, Avenoso A, Campo GM, Scordo MG, Caputi AP, and Perucca E (1997) Effect of ketoconazole on the pharmacokinetics of imipramine and desipramine in healthy subjects. *Br J Clin Pharmacol* **43**:315–318.
- Spina E, Pollicino AM, Avenoso A, Campo GM, Perucca E, and Caputi AP (1993) Effect of fluvoxamine on the pharmacokinetics of imipramine and desipramine in healthy subjects. *Ther Drug Monit* **15**:243–246.
- Steiner E and Spina E (1987) Differences in the inhibitory effect of cimetidine on desipramine metabolism between rapid and slow debrisoquin hydroxylators. *Clin Pharmacol Ther* **42**:278–282.
- Todor I, Popa A, Neag M, Muntean D, Bocsan C, Buzoianu A, Vlase L, Gheldiu AM, Chira R, and Briciu C (2015) The influence of paroxetine on the pharmacokinetics of atomoxetine and its main metabolite. *Clujul Med* **88**:513–520.
- Touche MA and Slaughter RL (1991) The effect of renal failure on hepatic drug clearance. *DIAP* **25**:1214–1224.
- Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang SM, and Sinha V (2015) Application of physiologically based pharmacokinetic (PBPK) modeling to support dose selection: report of an FDA public workshop on PBPK. *CPT Pharmacometrics Syst Pharmacol* **4**:226–230.
- Yang J, Jamei M, Yeo KR, Tucker GT, and Rostami-Hodjegan A (2007) Prediction of intestinal first-pass drug metabolism. *Curr Drug Metab* **8**:676–684.
- Zhao P, Rowland M, and Huang SM (2012) Best practice in the use of physiologically based pharmacokinetic modeling and simulation to address clinical pharmacology regulatory questions. *Clin Pharmacol Ther* **92**:17–20.

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