Special Section on Prediction of Human Pharmacokinetic Parameters from In Vitro Systems

CYP2B6 Pharmacogenetics–Based In Vitro–In Vivo Extrapolation of Efavirenz Clearance by Physiologically Based Pharmacokinetic Modeling[§]

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ABSTRACT

Efavirenz is mainly cleared by CYP2B6. The CYP2B6*6 allele is associated with lower efavirenz clearance. Efavirenz clearance was predictable using in vitro data for carriers of the CYP2B6*1/*1 genotype, but the prediction in carriers of the CYP2B6*6 allele was poor. To test the hypothesis that incorporation of mechanism of reduced efavirenz metabolism by the CYP2B6*6 allele can predict the genetic effect on efavirenz pharmacokinetics, in vitro–in vivo extrapolation of efavirenz clearance was performed by physiologically based pharmacokinetic modeling (Simcyp Simulator; Simcyp Ltd., Sheffield, UK) using data obtained from expressed CYP2B6.1 and CYP2B6.6 as well as human liver microsomes (HLMs) with CYP2B6*1/*1, *1/*6, and *6/*6 genotypes. Simulated pharmacokinetics of a single 600-mg oral dose of efavirenz for individuals with each genotype was compared with data observed in healthy subjects

genotyped for the CYP2B6*6 allele ($n = 20$). Efavirenz clearance for carriers of the CYP2B6*1/*1 genotype was predicted reasonably well using HLM data, but the clearance in carriers of the CYP2B6*6 allele was underpredicted using both expressed and HLM systems. Improved prediction of efavirenz clearance was obtained from expressed CYP2B6 after recalculating intersystem extrapolation factors for CYP2B6.1 and CYP2B6.6 based on in vitro intrinsic clearance of bupropion 4-hydroxylation. These findings suggest that genetic effect on both CYP2B6 protein expression and catalytic efficiency needs to be taken into account for the prediction of pharmacokinetics in individuals carrying the CYP2B6*6/*6 genotype. Expressed CYP2B6 proteins may be a reliable in vitro system to predict effect of the CYP2B6*6 allele on the metabolism of CYP2B6 substrates.

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Introduction

The clinical link between CYP2B6 metabolic status and drug disposition became clearer after the identification of CYP2B6 as the main clearance mechanism of efavirenz (Ward et al., 2003). The major clearance pathway of efavirenz is 8-hydroxylation, which is catalyzed predominantly by CYP2B6 with minor contributions from CYP1A2, CYP2A6, and CYP3A4/5 (Ward et al., 2003). CYP2A6-catalyzed 7-hydroxylation and UDP-glucuronosyltransferase (UGT) 2B7-catalyzed N-glucuronidation represent minor metabolic pathways (Belanger et al., 2009; Ogburn et al., 2010; Cho et al., 2011). Several in vitro studies indicate that CYP2B6 expression and activity are highly variable among individuals, which is in part due to polymorphisms in the CYP2B6 gene and nongenetic factors, e.g., induction and inhibition drug interactions (Turpeinen et al., 2006; Zanger et al., 2007; Wang and Tompkins, 2008).

ABBREVIATIONS: AUC, area under the plasma concentration-time curve; AUC_{0–∞}, area under the plasma drug concentration-time curve from zero to infinity; Cl_{int}, intrinsic clearance; CL_{ISEF}, ISEF values determined on the basis of Cl_{int}; CL_{po}, oral clearance; f_{umic}, fraction unbound in microsomes; HLM, human liver microsome; ISEF, intersystem extrapolation factor; IVIVE, in vitro–in vivo extrapolation; K_m, Michaelis-Menten constant; MRS, mean residual sum; 7-OHEFV, 7-hydroxyefavirenz; 8-OHEFV, 8-hydroxyefavirenz; PBPK, physiologically based pharmacokinetic model; rhCYP, recombinant human CYP proteins; RMSE, root mean square error; UGT, UDP-glucuronosyltransferase; VISEF, ISEF values determined on the basis of V_{max}.

Efavirenz, a cornerstone of anti-human immunodeficiency virus therapy, has a narrow therapeutic range, and its use is compromised by a large interpatient variability in exposure (up to 90-fold) (Marzolini et al., 2001; Csajka et al., 2003; Rotger et al., 2007). Using CYP2B6 genetic variations as markers, several clinical studies have demonstrated that differences in efavirenz clearance and responses are significantly influenced by interpatient differences in CYP2B6 activity. CYP2B6 is also an important determinant for drugs with a narrow therapeutic range, such as cyclophosphamide, methadone, nevirapine, and bupropion (Zanger et al., 2007). In this context, not only is efavirenz an in vitro and in vivo activity probe, but it can also serve as a prototype model drug for evaluating the clinical relevance of *CYP2B6* genetic polymorphisms and nongenetic factors influencing CYP2B6 activity.

In vitro–in vivo extrapolation (IVIVE) is a widely used approach to predict human drug metabolism, potential metabolism-mediated drug interactions, and genotype-phenotype associations from in vitro systems. Rekić et al. (2011) applied this approach to predict the induction effect of rifampicin on efavirenz steady-state pharmacokinetics considering weight and CYP2B6 phenotype as covariates. However, Rekić et al. did not address the specific genotype. Of all the CYP2B6 variants identified, the *CYP2B6**6 haplotype (516G>T and 785A>G) is by far the most frequent and functionally relevant variant across populations (Zanger et al., 2007). The *CYP2B6**6 allele is associated with reduced efavirenz clearance (Tsuchiya et al., 2004; Rotger et al., 2007), increased adverse effects (Haas et al., 2004; Yimer et al., 2012), and treatment discontinuation (Wyen et al., 2011). The mechanisms underlying the altered substrate metabolism by this variant allele have been explored in a series of studies: 1) reduced protein amount in human liver microsomes (HLMs) (Desta et al., 2007a), probably due to the aberrant splicing of pre-mRNA of CYP2B6 (Lamba et al., 2003; Hofmann et al., 2008); and 2) altered substrate binding and catalytic activity in vitro (Xu et al., 2012). The mechanism underlying the markedly reduced efavirenz metabolism by the *CYP2B6**6 allele in vitro (Desta et al., 2007a; Ariyoshi et al., 2011; Zhang et al., 2011; Xu et al., 2012) and in vivo (Tsuchiya et al., 2004) may involve one or both of these mechanisms. Recently, limited success in the prediction of efavirenz clearance for CYP2B6 slow metabolizers (*6/*6 genotype carriers) was reported (Siccardi et al., 2012). This may be partly due to the fact that the model in that study did not account for the changes in catalytic properties of the genetic variants compared with wild type. Thus, the modeling effort of the present study focused on the *CYP2B6**6 allele. In addition, Rekić et al. (2011) only tested the predictability of expressed cytochrome P450s. The present study aimed to systematically evaluate the utility of both genotyped HLMs and expressed CYP2B6 in predicting the effect of the *CYP2B6**6 allele on CYP2B6 activity in vivo using single-dose efavirenz as probe. Finally, efavirenz is known for its autoinduction of metabolism (Zhu et al., 2009). Recent study suggests that the *CYP2B6* genotype may alter the effect of rifampicin on the efavirenz autoinduction process (Ngaimisi et al., 2011). Therefore, in contrast to the study by Rekić et al. (2011) in which both single-dose and steady-state pharmacokinetics were simulated, only single-dose efavirenz pharmacokinetics was studied in the present study to avoid the confounding effect of *CYP2B6* genetics on the evaluation of predictability of two in vitro systems.

The first purpose of the present study was to carry out pharmacogenetics-based IVIVE of efavirenz exposure after a single 600-mg oral dose of efavirenz in healthy volunteers. The pharmacogenetics-based IVIVE was performed using in vitro efavirenz kinetic data generated from two systems (expressed CYP2B6.1 and CYP2B6.6 proteins as well as HLMs derived from liver tissues genotyped for the *CYP2B6**6 allele). The

second purpose of this study was to evaluate the predictability of two enzyme sources.

Materials and Methods

In Vitro Determination of Efavirenz Intrinsic Clearance in Expressed CYP2B6 and HLMs. In expressed CYP2B6.1 and CYP2B6.6 proteins, the kinetics for the formation of 8-hydroxyefavirenz was determined as reported in our previous publication (Xu et al., 2012). Briefly, 8-hydroxyefavirenz formed from efavirenz incubations in expressed CYP2B6.1 and CYP2B6.6 was quantified by a high-performance liquid chromatography/UV system (Xu et al., 2012). CYP2B6.1 and CYP2B6.6 proteins with coexpression of human cytochrome P450 oxidoreductase and plasmid-transfected negative controls were obtained from BD Biosciences (Woburn, MA) and provided by Dr. Guo (Eli Lilly and Company, Indianapolis, IN). To calculate the total in vitro intrinsic clearance (Cl_{int}) of efavirenz, the formation of 7- and 8-hydroxyefavirenz in HLMs obtained from liver tissues genotyped for the *CYP2B6**6 allele was simultaneously quantified by a liquid chromatography/tandem mass spectrometry method described in our previous publication (Ogburn et al., 2010). HLMs were prepared from human liver tissues by Eli Lilly and Company obtained from the Medical College of Wisconsin (Milwaukee, WI), Medical College of Virginia (Richmond, VA), Indiana University School of Medicine (Indianapolis, IN), and University of Pittsburgh (Pittsburgh, PA) under protocols approved by the appropriate committees for the conduct of human research, and provided by Dr. Guo.

Physiologically Based Pharmacokinetic Modeling. IVIVE of efavirenz exposure after a single 600-mg oral dose in healthy volunteers was performed using the Simcyp Population-based ADME Simulator (version 11.00; Simcyp Ltd.). The algorithm, physiologic basis, and differential equations used by the software have been described in previous publications (Rostami-Hodjegan and Tucker, 2007; Jamei et al., 2009). Input parameters including the physicochemical properties of efavirenz, in vitro Cl_{int} in HLMs, and expressed enzymes are listed in Table 1. All other parameters were set to default Simcyp values. Although UGT2B7 has been suggested to be involved in efavirenz metabolism in vitro (Belanger et al., 2009) and in vivo (Kwara et al., 2009), the contribution of the N-glucuronidation pathway to efavirenz overall metabolism seems minimal (Cho et al., 2011). Therefore, the present study did not take the UGT2B7-mediated pathway into account, because the contribution of this pathway to the overall efavirenz clearance is marginal. The virtual population was based on the built-in Simcyp healthy volunteer population and matched to the clinical study participants for number of subjects, age range, sex, and ethnicity. Twenty trials were simulated, and the overall mean and standard deviations of predicted pharmacokinetic parameters were compared with the observed values. Simulations were performed for an exclusive CYP2B6 extensive metabolizer population and an exclusive CYP2B6 poor metabolizer population. Initially, all combinations of the three models for absorption and two models for distribution, i.e., minimal and full physiologically based pharmacokinetic (PBPK) models implemented in the Simcyp, were tested. The abundance of CYP2B6 in *CYP2B6**1/*6 carriers was assumed to be the same as that in *1/*1 carriers (extensive metabolizers), i.e., 17 pmol/mg protein. Simulated efavirenz time-concentration profiles were compared with the observed plasma concentrations obtained from the clinical trial. The final model was selected based on visual predictive checks and comparison between the simulated and observed pharmacokinetic parameters [C_{max} , T_{max} (time to reach peak plasma concentration), area under the plasma concentration-time curve ($AUC_{0-\infty}$), and oral clearance (CL_{po})].

Recalculation of Intersystem Extrapolation Factors. An intersystem extrapolation factor (ISEF), a scaling factor to convert data obtained from an expressed cytochrome P450 system to HLMs by correcting for any difference in intrinsic enzyme activity between HLMs and expressed enzyme, was used (Proctor et al., 2004). ISEFs were defined with respect to either the V_{max} of metabolite formation of a probe substrate or Cl_{int} using eqs. 1 and 2:

$$VISEF = \frac{V_{max}(HLM)}{V_{max}(rhCYP) \times CYP \text{ abundance (HLM)}} \quad (1)$$

$$CLISEF = \frac{Cl_{int}(HLM)}{Cl_{int}(rhCYP) \times CYP \text{ abundance (HLM)}} \quad (2)$$

TABLE 1
In vitro input parameters of efavirenz

Physicochemical	Value	Reference	CL _{int} from HLMs (Pathway)		CL _{int} from rhCYP (Pathway)	
			μl/min/mg protein	μl/min/mg protein	μl/min/pmol P450	μl/min/pmol P450
MW	315.67		CYP2B6*1/*1 (8-OH) ^d	14.5	CYP2B6.1 (8-OH) ^a	0.39
LogP	4.6	(Siccardi et al., 2012)	CYP2B6*1/*6 (8-OH) ^d	6.4	CYP2B6.6 (8-OH) ^a	0.19
B/P ratio	0.74	(Balani et al., 1999)	CYP2B6*6/*6 (8-OH) ^d	2.4	CYP2A6 (7-OH) ^b	0.05
f _{u,plasma}	0.029	(Shou et al., 2008)	CYP2B6*1/*1 (7-OH) ^c	1.1	CYP2A6 (8-OH) ^b	0.08
f _{umic}	0.30	(Rekic et al., 2011)	CYP2B6*1/*6 (7-OH) ^c	1.5	CYP3A4 (8-OH) ^d	0.007
Plasma binding protein	Albumin		CYP2B6*6/*6 (7-OH) ^c	1.3	CYP3A5 (8-OH) ^d	0.03
Caco-2 permeability (10 ⁻⁵ cm/s)	8.92	(Takano et al., 2006)			CYP1A2 (8-OH) ^d	0.07

^aData from Xu, et al. (2012).

^bData from Ogburn, et al. (2010).

^cData from the present study.

^dData from Ward, et al. (2003).

where VISEF is the ISEF value determined on the basis of $V_{\max}$ and CLISEF is the ISEF value determined on the basis of CL_{int} . ISEFs built in Simcyp were calculated on the basis of $V_{\max}$ for each individual cytochrome P450. The default values of ISEFs for BD Biosciences CYP2B6 supersomes are 0.43, and were initially tested in our model. However, ISEF values may vary widely depending on the cytochrome P450 expression system, HLM preparations, probe substrate selected, and/or assay conditions used in each laboratory (Proctor et al., 2004). Therefore, we calculated the ISEF values for CYP2B6.1 and CYP2B6.6 based on $V_{\max}$ and CL_{int} values of bupropion 4-hydroxylation determined in HLMs with CYP2B6*1/*1 and *6/*6 genotypes and expressed CYP2B6 proteins from our previous study (Xu et al., 2012). The CYP2B6 abundance was assumed to be 17 pmol/mg and 6 pmol/mg in the wild type and homozygote according to Simcyp. The same simulations were performed as described earlier to evaluate the effect of different ISEF values on the prediction of efavirenz CL_{po} .

Clinical Trial. Efavirenz plasma concentrations were obtained from a pharmacokinetic study that was conducted as part of a drug-drug interaction trial by our group (Desta et al., 2007b). The study was approved by the Indiana University–Purdue University Indianapolis Institutional Review Board. Written, informed consent was obtained from each subject. The trial was a randomized, placebo-controlled crossover study. A total of 20 healthy subjects were recruited; 10 subjects were male and 10 subjects were female. All subjects were genotyped for the CYP2B6*6 allele and received a single 600-mg oral dose of efavirenz on an empty stomach after 10-day treatment with placebo or 600-mg oral dose of rifampicin. Blood samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, and 72 hours after efavirenz dosing for pharmacokinetic analysis. Plasma samples were separated by centrifugation at 3000 rpm for 20 minutes within an hour of blood collection and stored at -80°C until analysis. Plasma concentrations of efavirenz and its metabolites were determined by a validated liquid chromatography/tandem mass spectrometry method described previously (Ogburn et al., 2010). All pharmacokinetic parameters ($C_{\max}$, $T_{\max}$, $AUC_{0-\infty}$, and CL_{po}) were calculated by noncompartmental analysis using WinNonlin (version 5.01; Pharsight, Mountain View, CA). Only data from the placebo arm were used for the present study.

Statistical Analysis. Root mean square error (RMSE) and mean residual sum (MRS) were calculated based on eqs. 3 and 4 (Chen et al., 2011). A smaller RMSE indicates less variability between the prediction and observation. The MRS was used to measure the bias of different methods. A negative value indicates underestimation and a positive value indicates overestimation.

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (\text{predicted CL} - \text{observed CL})^2}{n}} \quad (3)$$

$$\text{MRS} = \frac{\sum_{i=1}^n (\text{predicted CL} - \text{observed CL})}{n} \quad (4)$$

The overall accuracies were determined by eq. 5 (Obach et al., 1997):

$$\text{Average fold error} = 10^{\left| \frac{\sum \log\left(\frac{\text{predicted}}{\text{observed}}\right)}{n} \right|} \quad (5)$$

Results

In Vitro Metabolism Data of Efavirenz. In two HLMs (HH-488, a *1/*6 carrier, and HH-478, a *6/*6 carrier), incubation of efavirenz did not produce quantifiable 7-hydroxyefavirenz (Supplemental Table 1). Kinetics for the formation of 7-hydroxyefavirenz were determined in a total of 13 HLM samples. A single-site Michaelis-Menten equation to estimate kinetic parameters ($V_{\max}$ and K_m) was fit to the formation rate of 7-hydroxyefavirenz for each HLM. The mean CL_{int} values of 7-hydroxylation calculated from HLMs with the same genotype are shown in Table 1. The mean CL_{int} values for the formation of 8-hydroxyefavirenz in expressed CYP2B6 and HLMs reported in our recent publication were used in the present simulation (Xu et al., 2012).

Efavirenz Single-Dose Pharmacokinetics. The pharmacokinetic parameters following a 600-mg oral dose of efavirenz are summarized in Table 2 and are consistent with a previous study (Haas et al., 2009). Efavirenz CL_{po} for individuals with CYP2B6*1/*1 ($n = 8$) and CYP2B6*1/*6 ($n = 9$) were 8.5 ± 3.4 and 8.3 ± 2.8 l/h, respectively. Correspondingly, the $AUC_{0-\infty}$ were 79.8 ± 28.4 and 81.6 ± 33.7 mg · h/l, respectively. Efavirenz CL_{po} for individuals with CYP2B6*6/*6 ($n = 3$) was 5.9 ± 0.5 l/h and the $AUC_{0-\infty}$ was 101.7 ± 7.9 mg · h/l. The difference in the CL_{po} values between individuals with CYP2B6*1/*1 and *1/*6 and those with CYP2B6*6/*6 did not reach statistical significance ($P = 0.23$). Based on the current data, 19 subjects with the CYP2B6*1/*1 and *1/*6 and 19 subjects with the CYP2B6*6/*6 genotype will be required to reach statistical significance of 5% with a power of 80%. We believe that the lack of statistical significance in the present study is most likely due to the inadequate number of subjects with the CYP2B6*6/*6 genotype. Individuals carrying the CYP2B6*6/*6 genotype were associated with a 30% reduction in CL_{po} and 27% increase in $AUC_{0-\infty}$ compared with individuals carrying the CYP2B6*1/*1 and *1/*6 genotype.

Prediction of Efavirenz Single-Dose Pharmacokinetics Using HLM CL_{int} by PBPK Modeling. A compartmental absorption transit model with a full PBPK model was selected to perform simulations for individuals carrying different genotypes based on the parameter estimations ($C_{\max}$, $T_{\max}$, $AUC_{0-\infty}$, and CL_{po}) and visual predictive checks. The comparisons of simulated mean, 5th, and 95th percentiles of efavirenz plasma concentration-time profiles for individuals with CYP2B6*1/*1, *1/*6, and *6/*6 genotypes using in vitro CL_{int} determined in HLMs and observed plasma concentrations are shown in

TABLE 2

Observed and simulated pharmacokinetic parameters for a single 600-mg oral dose of efavirenz in 20 healthy subjects by PBPK modeling

		Pharmacokinetic Parameters			
		$T_{\max}$	$C_{\max}$	$AUC_{0-\infty}$	CL_{po}
		<i>h</i>	<i>mg/l</i>	<i>mg · h/l</i>	<i>l/h</i>
Observed	<i>CYP2B6</i> *1/*1 (<i>n</i> = 8)	2.3 ± 1.0	2.3 ± 0.7	79.8 ± 28.4	8.5 ± 3.4
Predicted	<i>CYP2B6</i> *1/*1 HLM	2.0 ± 0.4	1.8 ± 0.4	75.6 ± 43.1	8.5 ± 6.9
Predicted	<i>CYP2B6</i> .1(Simcyp)	2.0 ± 0.4	1.8 ± 0.4	175.1 ± 83.5	4.1 ± 2.2
Predicted	<i>CYP2B6</i> .1 (VISEF)	2.0 ± 0.4	1.8 ± 0.4	84.3 ± 57.2	9.8 ± 6.9
Predicted	<i>CYP2B6</i> .1 (CLISEF)	1.9 ± 0.3	1.6 ± 0.4	39.0 ± 35.2	24.3 ± 20.8
Observed	<i>CYP2B6</i> *1/*6 (<i>n</i> = 9)	2.6 ± 1.7	1.7 ± 0.5	81.6 ± 33.7	8.3 ± 2.8
Predicted	<i>CYP2B6</i> *1/*6 HLM	2.1 ± 0.4	2.0 ± 0.3	165.8 ± 94.1	4.7 ± 3.2
Observed	<i>CYP2B6</i> *6/*6 (<i>n</i> = 3)	2.7 ± 1.5	2.4 ± 0.2	101.7 ± 7.9	5.9 ± 0.5
Predicted	<i>CYP2B6</i> *6/*6 HLM	2.1 ± 0.4	2.2 ± 0.3	305.1 ± 135.8	2.2 ± 1.4
Predicted	<i>CYP2B6</i> .6 (Simcyp)	2.1 ± 0.4	1.9 ± 0.3	235.9 ± 98.8	2.8 ± 1.6
Predicted	<i>CYP2B6</i> .6 (VISEF)	2.0 ± 0.4	2.0 ± 0.4	136.0 ± 83.8	6.6 ± 6.4
Predicted	<i>CYP2B6</i> .6 (CLISEF)	2.0 ± 0.4	2.1 ± 0.4	183.7 ± 93.3	4.2 ± 3.1

All values are presented as the mean ± S.D.

Fig. 1. The corresponding simulated and observed pharmacokinetic parameters are summarized in Table 2. About 85%, 97%, and 95% of the measured efavirenz plasma concentrations were within the 5th to 95th percentile of simulated concentrations for individuals with *CYP2B6**1/*1 (Fig. 1A), *CYP2B6**1/*6 (Fig. 1B), and *CYP2B6**6/*6 (Fig. 1C) genotypes, respectively.

For individuals with the *CYP2B6**1/*1 genotype, predicted mean values of $C_{\max}$, $T_{\max}$, $AUC_{0-\infty}$, and CL_{po} were in good agreement with observed values (Table 2). However, the PBPK model underpredicted efavirenz CL_{po} for individuals with the *CYP2B6**1/*6 (simulated versus observed: 4.7 ± 3.2 versus 8.3 ± 2.8 l/h) and *6/*6 (simulated versus observed: 2.2 ± 1.4 versus 5.9 ± 0.5 l/h) genotypes using HLM data. The predicted mean of CL_{po} for individuals with *CYP2B6**1/*6 and *CYP2B6**6/*6 was 43% and 63% lower than that obtained from the clinical trial, respectively. Accordingly, the predicted mean $AUC_{0-\infty}$ for individuals with *CYP2B6**1/*6 and *CYP2B6**6/*6 genotypes was 103.2% and 200.0% higher than the observed values, respectively. Individuals with the *CYP2B6**6/*6

genotype were predicted to have a 74% reduction in mean CL_{po} compared with individuals with the *CYP2B6**1/*1 genotype, whereas a smaller extent of reduction (~30%) was observed in the clinical trial.

Prediction of Efavirenz Single-Dose Pharmacokinetics Using Expressed CYP2B6 Cl_{int} by PBPK Modeling. The comparisons of simulated mean, 5th, and 95th percentiles of efavirenz concentration-time profiles for individuals with *CYP2B6**1/*1 and *CYP2B6**6/*6 using in vitro Cl_{int} determined in expressed CYP2B6.1 and CYP2B6.6 versus observed efavirenz plasma concentrations are shown in Fig. 2. The corresponding simulated and observed pharmacokinetic parameters are summarized in Table 2. About 87% and 90% of the measured efavirenz plasma concentrations were within the 5th to 95th percentile of simulated concentrations for individuals with *CYP2B6**1/*1 (Fig. 2A) and *CYP2B6**6/*6 (Fig. 2B) genotypes, respectively. The PBPK model predicted the mean values of $C_{\max}$ and $T_{\max}$ for individuals with *CYP2B6**1/*1 and *CYP2B6**6/*6 reasonably well. However, it underpredicted efavirenz mean CL_{po} for individuals with *CYP2B6**1/*1 (simulated versus observed: 4.1 ± 2.2 versus 8.5 ± 3.4 l/h) and

Fig. 1. Observed and PBPK-simulated efavirenz (EFV) concentration-time profiles after a single 600-mg oral dose of efavirenz in individuals with *CYP2B6**1/*1 (A), *CYP2B6**1/*6 (B), and *CYP2B6**6/*6 (C) genotypes using in vitro Cl_{int} determined in HLMs obtained from liver tissues with corresponding *CYP2B6**6 genotypes. Symbols represent the observed efavirenz plasma concentrations (0–72 hours) from healthy volunteers after a single 600-mg oral dose of efavirenz with *CYP2B6**1/*1 (*n* = 8), *CYP2B6**1/*6 (*n* = 9), and *CYP2B6**6/*6 (*n* = 3) genotypes. The solid lines represent the mean of simulated efavirenz plasma concentrations, whereas the dashed lines represent the 5th and 95th percentiles of simulated efavirenz plasma concentrations.

Fig. 2. Observed and PBPK-simulated efavirenz (EFV) concentration-time profiles after a single 600-mg oral dose of efavirenz in individuals with *CYP2B6**1/*1 (A) and *CYP2B6**6/*6 (B) genotypes using in vitro Cl_{int} generated from expressed CYP2B6.1 and CYP2B6.6 proteins (Simcyp ISEFs). Symbols represent the observed efavirenz plasma concentrations (0–72 hours) from healthy volunteers after a single 600-mg oral dose of efavirenz with *CYP2B6**1/*1 ($n = 8$) and *CYP2B6**6/*6 ($n = 3$) genotypes. The solid lines represent the mean of simulated efavirenz plasma concentrations, whereas the dashed lines represent the 5th and 95th percentiles of simulated efavirenz plasma concentrations.

*CYP2B6**6/*6 (simulated versus observed: 2.8 ± 1.6 l/h versus 5.9 ± 0.5 l/h) genotypes (Table 2). The predicted mean of CL_{po} for individuals with *CYP2B6**1/*1 and *CYP2B6**6/*6 genotypes was 52% and 53% lower than that observed in the clinical trial, respectively. Accordingly, the predicted mean of $AUC_{0-\infty}$ for individuals with *CYP2B6**1/*1 and *CYP2B6**6/*6 genotypes was 119.4% and 132.0% higher than that observed in the clinical trial, respectively. Individuals with the *CYP2B6**6/*6 genotype were predicted to have a 32% reduction in mean CL_{po} compared with individuals with *CYP2B6**1/*1.

Comparison of Prediction for Efavirenz Mean CL_{po} Using Simcyp Default and Recalculated ISEFs. ISEF values may vary widely depending on the cytochrome P450 expression system, HLM preparations, probe substrate selected, and/or assay conditions used in each laboratory (Proctor et al., 2004). Thus, the underprediction of CL_{po} using in vitro Cl_{int} in expressed CYP2B6 may indicate that the default settings of ISEFs for CYP2B6 in Simcyp may not apply to our system. Therefore, ISEFs of expressed CYP2B6.1 and CYP2B6.6 were calculated on the basis of V_{max} and Cl_{int} estimated from the kinetics of bupropion 4-hydroxylation determined in the HLMs and expressed cytochrome P450s from our previous publication (Xu et al., 2012). VISEF and CLISEF for CYP2B6 are shown in Table 3. For CYP2B6.1, the VISEF value was 1.9 and the CLISEF value was 6.5. For CYP2B6.6, the VISEF value was 1.6 and the CLISEF value was 0.9. The same simulations were performed as described earlier to evaluate the effect of different ISEF values on the prediction of

efavirenz CL_{po} . The simulated key pharmacokinetic parameters are summarized in Table 2. The predicted population median, 5th, and 95th percentiles (using Cl_{int} determined in expressed CYP2B6.1 and CYP2B6.6 correcting for Simcyp ISEF, recalculated VISEF and CLISEF) versus observed CL_{po} are shown in Fig. 3. For expressed CYP2B6.1, the prediction using VISEF reasonably captured the observed values of efavirenz CL_{po} , whereas underprediction using Simcyp ISEFs and overprediction using CLISEF was observed. For expressed CYP2B6.6, simulations using recalculated VISEF and CLISEF improved the prediction of efavirenz CL_{po} for individuals with the *CYP2B6**6/*6 genotype.

Comparison of Predictions of Efavirenz Mean CL_{po} by HLM and Expressed Systems. The performances of efavirenz clearance using HLM and expressed systems were evaluated by comparing predicted with observed efavirenz mean CL_{po} (Table 4). Predictions using expressed CYP2B6 corrected for VISEFs showed best prediction in terms of accuracy and variability.

Discussion

In the present study, we have successfully conducted a pharmacogenetics-based IVIVE of efavirenz clearance by a full-PBPK model. Efavirenz clearance for *CYP2B6**1/*1 carriers was predicted reasonably well using HLM data, but the clearance in carriers of the *CYP2B6**6 allele was underpredicted using both expressed and HLM systems. Predictions from expressed CYP2B6 were improved by recalculating ISEFs for CYP2B6.1 and CYP2B6.6 based on in vitro V_{max} and/or Cl_{int} of bupropion 4-hydroxylation, highlighting the significance of establishing ISEFs for wild-type and variant proteins in pharmacogenetics-based IVIVE of drug clearance. Based on these results, the potential utility of CYP2B6-expressed systems in pharmacogenetics-based IVIVE of pharmacokinetics of CYP2B6 substrates is suggested. We also provided new information that reduced protein expression associated with the *CYP2B6**6 allele and

TABLE 3
Recalculation of ISEFs

	VISEF	CLISEF	Simcyp
CYP2B6.1	1.9	6.5	0.43
CYP2B6.6	1.6	0.9	NA

Fig. 3. Observed and PBPK-predicted efavirenz CL_{po} after a single 600-mg oral dose of efavirenz in individuals with *CYP2B6**1/*1 and *CYP2B6**6/*6 genotypes using in vitro Cl_{int} determined in expressed CYP2B6.1 and CYP2B6.6 correcting for different values of ISEFs. The simulations of CL_{po} were performed incorporating default values of ISEFs for expressed CYP2B6 built in the Simcyp, VISEF, and CLISEF of bupropion 4-hydroxylation. Horizontal black lines represent the 95th percentile, median, and 5th percentile of simulations. Symbols represent CL_{po} estimated from pharmacokinetics observed in the individuals with *CYP2B6**1/*1 ($n = 8$) and *CYP2B6**6/*6 ($n = 3$) in the clinical trial.

altered catalytic properties of the CYP2B6.6 protein contributes to reduced efavirenz metabolism in vitro and probably in vivo. Incorporation of these two mechanisms into pharmacogenetics-based IVIVE of clearance of efavirenz and other CYP2B6 substrates in individuals carrying the *CYP2B6**6 allele is needed.

HLMs are often considered to be more physiologically relevant than an expressed system, but their use is limited by inadequate availability, variable quality of tissues, and variability between donors (Hallifax et al., 2010). Indeed, there is a large variability in the catalytic velocities and in vitro Cl_{int} of efavirenz 8-hydroxylation in HLMs even within the same genotype (Desta et al., 2007a; Xu et al., 2012). Expressed cytochrome P450s have been used increasingly in the prediction of drug clearance due to their availability and simplicity. However, the

determination of Cl_{int} values for 8-hydroxylation has been shown to be dependent on the expressed systems (Ariyoshi et al., 2011; Zhang et al., 2011; Xu et al., 2012) and presence of cytochrome b5 (Xu et al., 2012). In the present study, we systematically evaluated the utility of data derived from expressed CYP2B6 proteins (CYP2B6.1 and CYP2B6.6) and HLMs with *CYP2B6**1/*1, *1/*6, and *6/*6 genotypes under the same experimental conditions in IVIVE of efavirenz clearance by PBPK modeling. We have shown that 1) kinetic data derived from both in vitro systems reliably predicted the oral clearance of a single 600-mg oral dose of efavirenz in carriers of the *CYP2B6**1/*1 genotype; 2) expressed CYP2B6 proteins better predicted efavirenz clearance in individuals with the *CYP2B6**6/*6 genotype by recalculating ISEFs for CYP2B6.1 and CYP2B6.6 based on in vitro kinetics of bupropion 4-hydroxylation than those derived from HLMs, which underpredicted clearance; and 3) HLMs underpredicted clearance in carriers of the *CYP2B6**1/*6 genotype. It appears that expressed CYP2B6 protein may be superior to HLMs in IVIVE, especially for the prediction in individuals with reduced-function alleles. However, variable successes were obtained using data from expressed cytochrome P450s, e.g., CYP2C9 (Dickinson et al., 2007a; Kusama et al., 2009), CYP2D6 (Dickinson et al., 2007b), and CYP2B6 (Siccardi et al., 2012). Therefore, the selection of a relevant in vitro system may be dependent on the specific cytochrome P450 isoform and variant allele studied.

Although the reasons for underprediction of clearance using HLMs in carriers of the *CYP2B6**6 allele are not fully understood, several factors could be suggested. First, although it seems unlikely, is the uncertainty in the determination of f_{umic} . Several predictive equations for f_{umic} determined by drug lipophilicity and protein concentrations have been developed (Austin et al., 2002; Hallifax and Houston, 2006; Turner et al., 2007). The protein concentrations used in HLM and CYP2B6 incubations were 0.25–1 mg/ml, and thus f_{umic} of efavirenz was estimated to be 0.2–0.4. A published f_{umic} value of 0.3 with reasonably good prediction of efavirenz clearance (Rekic et al., 2011) was contained in that range and therefore was used in the present study. The second possibility may be the additive effect of reduced protein expression by the *CYP2B6**6 allele on catalytic efficiency in HLM and overall extrapolated Cl_{int} . In vitro studies suggest that altered binding affinity and reduced catalytic efficiency secondary to reduced protein expression may contribute to reduced efavirenz metabolism in HLMs with the *CYP2B6**6/*6 genotype (Desta et al., 2007a; Xu et al., 2012). Indeed, the link between in vitro protein expression and in vivo function of CYP2B6 seems somewhat weak. For example, CYP2B6 protein expression is substantially reduced in

TABLE 4

Prediction of efavirenz mean CL_{po} from in vitro data generated in genotyped HLMs and expressed CYP2B6 by PBPK modeling

Simcyp ISEF values are Simcyp default ISEF values. RMSE, MRS, and average fold error were calculated by comparing the predicted efavirenz mean CL_{po} by PBPK modeling to observed mean values.

	Predicted Mean CL_{po}	Observed Mean CL_{po}	RMSE	MRS	Average Fold Error
HLMs					
<i>CYP2B6</i> *1/*1	8.5	8.5	3.0	−2.5	2.3
<i>CYP2B6</i> *1/*6	4.7	8.3			
<i>CYP2B6</i> *6/*6	2.1	5.9			
Expressed cytochrome P450s (Simcyp ISEF)			3.8	−3.8	3.2
<i>CYP2B6</i> *1/*1	4.1	8.5			
<i>CYP2B6</i> *6/*6	2.8	5.9			
Expressed cytochrome P450s (VISEF)			1.0	1.0	0.55
<i>CYP2B6</i> *1/*1	9.8	8.5			
<i>CYP2B6</i> *6/*6	6.6	5.9			
Expressed cytochrome P450s (CLISEF)			11.2	7.1	1.5
<i>CYP2B6</i> *1/*1	24.3	8.5			
<i>CYP2B6</i> *6/*6	4.2	5.9			

HLMs with the *CYP2B6**1/*6 genotype compared with those with the *CYP2B6**1/*1 genotype (Desta et al., 2007a), but the in vivo phenotypes of the *CYP2B6**1/*1 and *1/*6 carriers are close and sometimes overlap for substrates such as efavirenz, methadone, and cyclophosphamide (Tsuchiya et al., 2004; Crettol et al., 2005; Nakajima et al., 2007). Of note, the approximately 8-fold reduction in protein expression associated with the *CYP2B6**5/*5 genotype in vitro (Lang et al., 2001) did not translate into a significant effect in vivo for any of the substrates tested (Kirchheiner et al., 2003; Winoto et al., 2011).

These data may also imply that the change in K_m as a result of amino acid changes by the *CYP2B6**6 allele may be the predominant mechanism by which this allele alters the metabolism of CYP2B6 substrates. This implication is further supported by the fact that this allele has been associated with enhanced cyclophosphamide metabolism (Nakajima et al., 2007) in contrast to the reduced metabolism observed for efavirenz (Tsuchiya et al., 2004), nevirapine (Rotger et al., 2005), and methadone (Eap et al., 2007), which is primarily driven by a significantly lower K_m for cyclophosphamide 4-hydroxylation in CYP2B6.6 than in CYP2B6.1 (Ariyoshi et al., 2011). Therefore, we speculate that the overprediction of efavirenz clearance observed for individuals carrying the *CYP2B6**6/*6 genotype in a recent study could be due to the fact that the reduction in efavirenz binding affinity and catalytic efficiency by the *CYP2B6**6 allele was not taken into account in that model (Siccardi et al., 2012). The improved prediction of efavirenz clearance for individuals with the *CYP2B6**6/*6 genotype in the present study indicates the importance of considering altered catalytic properties of the *CYP2B6**6 allele in CYP2B6 pharmacogenetics-based IVIVE (Jinno et al., 2003; Watanabe et al., 2010; Ariyoshi et al., 2011; Zhang et al., 2011; Xu et al., 2012). We found that predictions from expressed CYP2B6 were improved by recalculating ISEFs for CYP2B6.1 and CYP2B6.6 based on in vitro kinetics of bupropion 4-hydroxylation, highlighting the significance of establishing ISEFs for wild-type and variant proteins. ISEF has been used to account for the difference in turnover number (activity per unit amount of P450) between expressed cytochrome P450s and HLMs (Proctor et al., 2004) and successfully used for IVIVE to predict human clearance in a number of studies (Howgate et al., 2006; Dickinson et al., 2007a,b; Siccardi et al., 2012). ISEFs have been shown to depend on the expressed systems, HLM preparations, substrates used, and assay conditions (Proctor et al., 2004). The ISEF values are determined based on enzyme abundance, K_m , and V_{max} (Proctor et al., 2004), all of which have been shown to be altered by the *CYP2B6**6 allele (Hofmann et al., 2008; Zhang et al., 2011; Xu et al., 2012). Therefore, it is important to test the effect of this allele on ISEFs of CYP2B6. Indeed, incorporation of recalculated ISEFs greatly improved the prediction of efavirenz clearance. The prediction performance of VISEF is generally better than CLISEF, which indicates that the difference in K_m determined from expressed CYP2B6 and HLMs may be marginal. These results highlight the importance of establishing ISEFs for an individual cytochrome P450 isoform and its variant proteins when general values of ISEFs do not perform well.

In summary, a full-PBPK model incorporating the effect of the *CYP2B6**6 allele on both CYP2B6 protein expression and catalytic properties successfully predicted the clearance after a single oral dose of efavirenz. Our data provide evidence that expressed CYP2B6 protein after recalculation of ISEFs may be superior to HLMs in IVIVE of efavirenz clearance. Due to the limited availability of well-characterized HLMs with specific genotype information, expressed CYP2B6 wild-type and variant proteins may be alternative and even preferred tools to determine the influence of pharmacogenetics on

substrate metabolism and drug interactions. Based on the data presented, we speculate that altered substrate binding due to the amino acid changes harbored in the variant protein coded by the *CYP2B6**6 allele may be the prominent mechanism for reduced efavirenz metabolism in vivo. The PBPK model developed in the present study can serve as a base model to predict single-dose and steady-state exposure of CYP2B6 substrates with linear pharmacokinetics, but further modification and validation may be needed in some situations. For example, the performance of this model in the prediction of efavirenz single-dose pharmacokinetics may be different from that of steady-state pharmacokinetics because efavirenz enhances its own clearance upon repeated administrations (Zhu et al., 2009) via upregulation of CYP2B6 (Faucette et al., 2007). In addition, the effect of the *CYP2B6**6 allele on metabolism seems to be substrate-dependent as discussed in the previous paragraph (Tsuchiya et al., 2004; Rotger et al., 2005; Eap et al., 2007; Nakajima et al., 2007). In conclusion, a pharmacogenetics-based PBPK model has been developed to predict efavirenz clearance after a single dose in a genotype-based manner. This model has the potential to facilitate the design of clinical pharmacokinetics/pharmacodynamics as well as drug-drug interaction studies of efavirenz and other CYP2B6 substrates.

Authorship Contributions

Participated in research design: Xu, Desta.

Conducted experiments: Xu.

Contributed new reagents or analytic tools: Quinney, Guo, Li, Desta.

Performed data analysis: Xu, Hall, Li, Desta.

Wrote or contributed to the writing of the manuscript: Xu, Quinney, Guo, Hall, Desta.

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