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.

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; C_{Lmin}, intrinsic clearance; CLISEF, ISEF values determined on the basis of C_{Lmin}; C_{Lpo}, oral clearance; f_{unb}, 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 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 (S16G>T and 785A>G) is by far the most frequent and functionally relevant variant across populations (Zanger et al., 2007). This may be partly due to the fact that the model in that study was selected based on visual predictive checks and comparison between the simulated and observed pharmacokinetic parameters obtained from the clinical trial. The final model 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 CYP2B6extensive 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 CYP2B6*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 |\(C_{\text{max}}\), \(T_{\text{max}}\) (time to reach peak plasma concentration), area under the plasma concentration-time curve (AUC\(_{0-\infty}\)), and oral clearance (CL\(_{\text{oral}}\)).

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 \(V_{\text{max}}\) of metabolite formation of a probe substrate or \(CL_{\text{int}}\) using eqs. 1 and 2:

\[
\text{VISEF} = \frac{V_{\text{max}}(\text{HLM})}{V_{\text{max}}(\text{rhCYP}) \times \text{CYP abundance (HLM)}}
\]

\[
\text{CLISEF} = \frac{\text{CLint (HLM)}}{\text{Clint (rhCYP)} \times \text{CYP abundance (HLM)}}
\]

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/UVM system (Xu et al., 2012). CYP2B6.1 and CYP2B6.6 proteins with coexpression of human cytochrome P450 450 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 (\(CL_{\text{int}}\)) 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.
where VISEF is the ISEF value determined on the basis of \( V_{\text{max}} \) and CLISEF is the ISEF value determined on the basis of \( C_{\text{int}} \). ISEFs built in Simcyp were calculated on the basis of \( V_{\text{max}} \) 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 assay conditions used in each laboratory (Proctor et al., 2004). Therefore, we calculated the ISEF values for CYP2B6.1 and CYP2B6.6 based on \( V_{\text{max}} \) and \( C_{\text{int}} \) 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 \( CL_{\text{po}} \).

**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 \(-80^\circ\text{C}\) 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 (\( C_{\text{max}}, T_{\text{max}}, \text{AUC}_{0-\infty}, \text{and } CL_{\text{po}} \)) 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.

\[
\text{RMSE} = \sqrt{\frac{\sum (\text{predicted } CL - \text{observed } CL)^2}{n}}
\]

\[
\text{MRS} = \frac{\sum (\text{predicted } CL - \text{observed } CL)}{n}
\]

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

\[
\text{Average fold error} = 10 \left| \frac{\log (\text{predicted } CL)}{\text{observed } CL} \right| 
\]

**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 (\( V_{\text{max}} \) and \( K_m \)) was fit to the formation rate of 7-hydroxyefavirenz for each HLM. The mean \( C_{\text{int}} \) values of 7-hydroxylation calculated from HLMs with the same genotype are shown in Table 1. The mean \( C_{\text{int}} \) 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 \( CL_{\text{po}} \) 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 \( \text{AUC}_{0-\infty} \) were 79.8 ± 28.4 and 81.6 ± 33.7 mg · h/l, respectively. Efavirenz \( CL_{\text{po}} \) for individuals with CYP2B6*6/*6 (\( n = 3 \)) was 5.9 ± 0.5 l/h and the \( \text{AUC}_{0-\infty} \) was 101.7 ± 7.9 mg · h/l. The difference in the \( CL_{\text{po}} \) 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 \( CL_{\text{po}} \) and 27% increase in \( \text{AUC}_{0-\infty} \) compared with individuals carrying the CYP2B6*1/*1 and *1/*6 genotype.

**Prediction of Efavirenz Single-Dose Pharmacokinetics Using HLM \( C_{\text{int}} \) 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 (\( C_{\text{max}}, T_{\text{max}}, \text{AUC}_{0-\infty}, \text{and } CL_{\text{po}} \)) 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 \( C_{\text{int}} \) determined in HLMs and observed plasma concentrations are shown in
Observed and simulated pharmacokinetic parameters for a single 600-mg oral dose of efavirenz in 20 healthy subjects by PBPK modeling

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (mg/l)</th>
<th>AUC&lt;sub&gt;0-&lt;i&gt;T&lt;/i&gt;&lt;/sub&gt; (mg h/l)</th>
<th>CL&lt;sub&gt;po&lt;/sub&gt; (l/h)</th>
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<tbody>
<tr>
<td>Observed</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CYP2B6*1/*1 (n = 8)</td>
<td>2.3 ± 1.0</td>
<td>2.3 ± 0.7</td>
<td>79.8 ± 28.4</td>
<td>8.5 ± 3.4</td>
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<tr>
<td>CYP2B6*1/*1 HLM</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>75.6 ± 43.1</td>
<td>8.5 ± 6.9</td>
</tr>
<tr>
<td>Predicted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2B6.1 (Simcyp)</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>175.1 ± 83.5</td>
<td>4.1 ± 2.2</td>
</tr>
<tr>
<td>CYP2B6.1 (VISEF)</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>84.3 ± 57.2</td>
<td>9.8 ± 6.9</td>
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<tr>
<td>CYP2B6*1/*6 (n = 9)</td>
<td>2.6 ± 1.7</td>
<td>1.7 ± 0.5</td>
<td>81.6 ± 33.7</td>
<td>8.3 ± 2.8</td>
</tr>
<tr>
<td>CYP2B6*1/*6 HLM</td>
<td>2.1 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>165.8 ± 94.1</td>
<td>4.7 ± 3.2</td>
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<tr>
<td>CYP2B6*6/*6 (n = 3)</td>
<td>2.7 ± 1.5</td>
<td>2.4 ± 0.2</td>
<td>101.7 ± 7.9</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>CYP2B6*6/*6 HLM</td>
<td>2.1 ± 0.4</td>
<td>2.2 ± 0.3</td>
<td>305.1 ± 135.8</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>CYP2B6*6/*6 (Simcyp)</td>
<td>2.1 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>235.9 ± 98.8</td>
<td>2.8 ± 1.6</td>
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<tr>
<td>CYP2B6*6/*6 (VISEF)</td>
<td>2.0 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>136.0 ± 83.8</td>
<td>6.6 ± 6.4</td>
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<tr>
<td>CYP2B6*6/*6 (CLISEF)</td>
<td>2.0 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>183.7 ± 93.3</td>
<td>4.2 ± 3.1</td>
</tr>
</tbody>
</table>

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

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<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-<i>T</i></sub>, and CL<sub>po</sub> were in good agreement with observed values (Table 2). However, the PBPK model underpredicted efavirenz mean CL<sub>po</sub> 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<sub>po</sub> 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<sub>0-<i>T</i></sub> 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<sub>po</sub> 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<sub>po</sub> 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<sub>int</sub> 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<sub>max</sub> and T<sub>max</sub> for individuals with CYP2B6*1/*1 and CYP2B6*6/*6 reasonably well. However, it underpredicted efavirenz mean CL<sub>po</sub> 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<sub>int</sub> 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.](image-url)
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 CLpo 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 AUC0- 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 CLpo compared with individuals with CYP2B6*1/*1.

**Comparison of Prediction for Efavirenz Mean CLpo 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 CLpo 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 AUC0- 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 CLpo compared with individuals with CYP2B6*1/*1.

**Comparison of Predictions of Efavirenz Mean CLpo by HLM and Expressed Systems.** The performances of efavirenz clearance using HLM and expressed systems were evaluated by comparing predicted with observed efavirenz mean CLpo (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 Vmax and/or CLint 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>
<thead>
<tr>
<th>TABLE 3 Recalculation of ISEFs</th>
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<tbody>
<tr>
<td>VISEF</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>CYP2B6.1</td>
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<tr>
<td>CYP2B6.6</td>
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**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 CLint 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.
individuals carrying the IVIVE of clearance of efavirenz and other CYP2B6 substrates in corporation of these two mechanisms into pharmacogenetics-based reduced efavirenz metabolism in vitro and probably in vivo. In-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 viability 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 the same genotype (Desta et al., 2007a; Xu et al., 2012). Expressed cytochrome P450s (Simcyp ISEF) 3.8 HLMs 3.0 Expressed cytochrome P450s (VISEF) 1.0 1.0 0.55 Expressed cytochrome P450s (CLISEF) 11.2 7.1 1.5 2.5 2.3 Predicted Mean Cl_{po} Observed Mean Cl_{po} RMSE MRS Average Fold Error

### TABLE 4

<table>
<thead>
<tr>
<th>HLMs</th>
<th>Predicted Mean Cl_{po}</th>
<th>Observed Mean Cl_{po}</th>
<th>RMSE</th>
<th>MRS</th>
<th>Average Fold Error</th>
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<tr>
<td>CYP2B6*1/*1</td>
<td>8.5</td>
<td>8.5</td>
<td>3.0</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>CYP2B6*1/*6</td>
<td>4.7</td>
<td>8.5</td>
<td>3.8</td>
<td>3.8</td>
<td>3.2</td>
</tr>
<tr>
<td>CYP2B6*6/*6</td>
<td>2.1</td>
<td>5.9</td>
<td>2.8</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Expressed cytochrome P450s (Simcyp ISEF)</td>
<td>4.1</td>
<td>8.5</td>
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<td>CYP2B6*1/*1</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>0.55</td>
</tr>
<tr>
<td>CYP2B6*1/*6</td>
<td>6.6</td>
<td>5.9</td>
<td>6.6</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Expressed cytochrome P450s (VISEF)</td>
<td>9.8</td>
<td>8.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2B6*1/*1</td>
<td>24.3</td>
<td>8.5</td>
<td>11.2</td>
<td>7.1</td>
<td>1.5</td>
</tr>
<tr>
<td>CYP2B6*1/*6</td>
<td>4.2</td>
<td>5.9</td>
<td></td>
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</tbody>
</table>

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_{univ}. Several predictive equations for f_{univ} 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_{univ} of efavirenz was estimated to be 0.2–0.4. A published f_{univ} 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 carriers of the CYP2B6*6/*6 genotype; 2) expressed CYP2B6 proteins better predicted efavirenz clearance in individuals with the CYP2B6*6/*6 genotype by re-calculation of 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.
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 (Tschiya 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 (Kirchheimer 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 (Tschiya 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 butropin 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 predominant 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 (Tschiya 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.

References

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