In Vivo Information-Guided Prediction Approach for Assessing the Risks of Drug-Drug Interactions Associated with Circulating Inhibitory Metabolites

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ABSTRACT:

The in vivo drug-drug interaction (DDI) risks associated with cytochrome P450 inhibitors that have circulating inhibitory metabolites cannot be accurately predicted by conventional in vitro-based methods. A novel approach, in vivo information-guided prediction (IVIP), was recently introduced for CYP3A- and CYP2D6-mediated DDIs. This technique should be applicable to the prediction of DDIs involving other important cytochrome P450 metabolic pathways. Therefore, the aims of this study were to extend the IVIP approach to CYP2C9-mediated DDIs and evaluate the IVIP approach for predicting DDIs associated with inhibitory metabolites. The analysis was based on data from reported DDIs in the literature. The IVIP approach was modified and extended to CYP2C9-mediated DDIs. Thereafter, the IVIP approach was evaluated for predicting the DDI risks of various inhibitors with inhibitory metabolites. Although the data on CYP2C9-mediated DDIs were limited compared with those for CYP3A- and CYP2D6-mediated DDIs, the modified IVIP approach successfully predicted CYP2C9-mediated DDIs. For the external validation set, the prediction accuracy for area under the plasma concentration-time curve (AUC) ratios ranged from 70 to 125%. The accuracy (75–128%) of the IVIP approach in predicting DDI risks of inhibitors with circulating inhibitory metabolites was more accurate than in vitro-based methods (28–80%). The IVIP model accommodates important confounding factors in the prediction of DDIs, which are difficult to handle using in vitro-based methods. In conclusion, the IVIP approach could be used to predict CYP2C9-mediated DDIs and is easily modified to incorporate the additive effect of circulating inhibitory metabolites.

Introduction

Drug-drug interactions (DDIs) can result when one drug alters the pharmacokinetics (PKs) of another drug or its metabolites. According to the new FDA Draft Guidance for Industry (2012, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf), the PK interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug’s safety and effectiveness. Therefore, predicting clinically significant drug interactions during drug development is essential for the pharmaceutical industry and regulatory agencies. The large number of clinically significant DDIs due to the inhibition of cytochrome P450 (P450) substrate metabolism and the availability of in vitro, in vivo, and clinical methods for assessing P450 DDIs have made this a logical starting point for the development and validation of techniques to predict clinically significant DDIs.

There exists a broad consensus as to the common principles underlying prediction of the magnitude of an in vivo DDI from in vitro data. The increase in the area under the plasma concentration-time curve (AUC) of a substrate when coadministered in the presence of a reversible inhibitor of the substrate’s elimination pathway is a function of the ratio of the inhibitor concentration ([I]) to inhibition constant (K_i) (Ito et al., 1998; Shou, 2005; Brown et al., 2006; Obach et al., 2006; Einfeld, 2007). A similar model involving K_i (concentration of inhibitor required to achieve half-maximal inactivation) and k_inact (maximal rate constant of enzyme inactivation) for DDIs associated with irreversible (mechanism-based) inhibitors has also been proposed (Obach et al., 2007). In addition, researchers have incorporated the fraction of substrate clearance mediated by the inhibited enzyme (f_{uncYP}), the plasma protein binding of the inhibitor (Shardlow et al., 2011), and fraction of absorbed substrate dose escaping gut metabolism by CYP3A (F_G) (Galetin et al., 2008) to improve predictions for certain drug classes.

Although in vitro-based models can quantitatively predict many in vivo DDIs with acceptable accuracy, the application of this model to the prediction of DDIs associated with P450 inhibitors that have inhibitory metabolites has not been successful (Yeung et al., 2011; McDonald et al., 2012). The prediction accuracy of in vitro models may be improved to some extent when data pertaining to metabolites

ABBREVIATIONS: DDI, drug-drug interaction; PK, pharmacokinetic; FDA, U.S. Food and Drug Administration; P450, cytochrome P450; AUC, area under the plasma concentration-time curve; IVIP, in vivo information-guided prediction; CR, contribution ratio; IR, the apparent inhibition ratio of the inhibiting drug; UHI, unbound hepatic inlet concentration.
were included in the model; however, prediction accuracy (35–188%) was still unsatisfactory for DDIs associated with 10 typical inhibitors that have inhibitory metabolites (Yeung et al., 2011). Another recent study found that prediction accuracy decreased when more inhibitory metabolites of amiodarone were taken into account (McDonald et al., 2012). A novel approach, in vivo information-guided prediction (IVIP), was recently introduced for CYP3A- and CYP2D6-mediated drug interactions (Ohno et al., 2007; Tod et al., 2011). This model relies primarily on in vivo data and uses two characteristic parameters: one for the substrate and the other for the inhibitor. This model has the potential to take into account inhibitory metabolites, different mechanisms of inhibition, and intestinal inhibition. Although information on the inhibitory metabolites can also be incorporated into an in vitro-based model, the IVIP approach has certain advantages compared with in vitro-based methods. Validation of the IVIP approach for the prediction of DDIs mediated by other CYP450 enzymes or the effects of inhibitory metabolites on DDIs is still lacking, because of the paucity of available data. Therefore, the aims of this study are to extend the IVIP approach to CYP2C9-mediated interactions and to validate the modified IVIP approach for prediction of DDIs associated with inhibitors that have inhibitory metabolites.

Materials and Methods

Extending the IVIP Approach to CYP2C9-Mediated Interactions. Medline, PubMed, and Embase databases (from 1975 until December 31, 2011) were searched using the terms “CYP2C9,” “inhibition,” and “pharmacokinetics.” Citations within the retrieved articles were used to search for additional relevant studies. Studies were included if 1) they were conducted in humans, 2) they provided the ratio between the AUC of the substrate when administered alone and when coadministered at the same dose with the inhibitor, 3) the dose of the inhibitor was within the therapeutic dose range, and 4) the inhibitor and substrate drugs were orally or intravenously administered to the subjects. Drug-drug interaction studies associated with herbal products, combination therapies, and oral contraceptives were excluded. Both reversible and irreversible inhibitors were included in the analysis.

An IVIP approach that has been described previously was modified and applied to the quantitative prediction of CYP2C9-mediated DDIs (Ohno et al., 2007; Tod et al., 2011). This modeling framework uses two characteristic parameters: the contribution ratio (CR) defined as the contribution of the specific enzyme to the oral clearance or total clearance (intravenous administration) of the drug whose metabolism is inhibited (victim) and the apparent inhibition ratio (IR) of the inhibiting drug (perpetrator). If reasonable estimates of CR (0 ≤ CR ≤ 1) and IR (0 ≤ IR ≤ 1) can be determined, then the ratio of the AUC of the inhibitor is coadministered. CR values of most CYP2C9 substrates were estimated by the extrapolation method (learning set 2), CR values were estimated by eq. 4 using the known IR of the inhibitors. An external validation of these estimates was performed by comparing the AUC ratios predicted by eq. 1 with the observed values from data not included in the preceding steps. An algebraic mean of the AUC increase was used in the calculation whenever results from multiple studies were available for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).

Evaluation of the IVIP Approach in Predicting the DDI Risks of Various Inhibitors with Circulating Inhibitory Metabolites. The relevant data of clinical DDIs associated with P450 inhibitors that have inhibitory metabolites were mainly retrieved from a single recent report (Yeung et al., 2011), which is based on the University of Washington Metabolism and Transport Drug Interaction Database (MTDI database: http://www.druginteractioninfo.org). Whenever available, additional data from the literature were included. In vivo DDI studies were included in our analysis only if they had been conducted with a reliable P450 probe. For CYP3A- and CYP2D6-mediated DDIs, only data from oral administration were included in the analysis. An algebraic mean of the AUC increase was used in the calculation when multiple studies were available for a single combination of victim drug and inhibitor (the same dose of inhibitor was used in these studies).

With use of the above retrieved data, in vivo DDIs associated with inhibitory metabolites were predicted by the fully validated IVIP approach. To avoid “self-prediction,” the data in the learning set were not included in the validation set and vice versa. The learning sets for CYP2D6- and CYP3A-mediated DDIs were selected according to the following criteria: 1) the dose of the inhibitor in the learning set is the same as in the validation set, 2) the regimens of the inhibitor in the learning set and the validation set are both multiple-dose or single-dose regimen, and 3) the victim drug is a known probe or a substrate with a relatively high CR value.

The CR values of most CYP2C9 substrates were estimated by the extrapolation method (CR = f_{\text{ext}}/f_{\text{int}}) using literature data. A learning set (learning set 1) was selected to calculate the IR of CYP2C9 inhibitors. Only the DDIs associated with typical CYP2C9 substrates (S-warfarin, tolbutamide, diclofenac, and phenytoin) were included in this learning set. For the inhibitors with different levels of doses, IR values were estimated for each dose because IR should be dose-dependent. For the remaining few substrates whose CR could not be calculated by the extrapolation method (learning set 2), CR values were estimated by eq. 4 using the known IR of the inhibitors. An external validation of these estimates was performed by comparing the AUC ratios predicted by eq. 1 with the observed values from data not included in the preceding steps.

\[
\text{CR} = \frac{\text{AUC}_{\text{int}}/\text{AUC}_{\text{ext}} - 1}{\text{AUC}_{\text{int}}/\text{AUC}_{\text{ext}}} \quad (3)
\]

Estimation of CR using the interaction method (eq. 4) is based on transformation of eq. 1 using a known IR value of the inhibitor (IR was assumed to be 1.0 for a very strong inhibitor), where AUC_{i} is the AUC of the drug when the inhibitor is coadministered.

\[
\text{CR} = \frac{\text{AUC}_{i}/\text{AUC} - 1}{\text{AUC}_{i}/\text{AUC} \cdot \text{IR}} \quad (4)
\]

However, the IVIP approach developed for CYP2D6- and CYP3A-mediated DDIs cannot be directly extended to CYP2C9-mediated DDIs without modification. The CR values of CYP2C9 substrates cannot be reliably estimated by the pharmacogenetic method because relevant studies in CYP2C9 poor metabolizers are limited. Furthermore, the CR of most CYP2C9 substrates cannot be calculated by eq. 4 because of the absence of IR data (e.g., the IR value can be assumed to be 1 for a very strong P450 inhibitor, but no strong inhibitor of CYP2C9 has been found according to the new FDA Draft Guidance for Industry and a recent study (Polasek et al., 2011). Therefore, the CR of CYP2C9 substrates was estimated using eq. 5, where f_{\text{ext}} is the contribution of CYP2C9 to hepatic clearance (estimated in vitro by CYP2C9-specific inhibitor or functional neutralizing antibody) and f_{\text{int}} is the contribution of the hepatic clearance to the total clearance of the drug (estimated by the recovery of excreted CYP2C9 metabolites in urine, bile, and feces).

\[
\text{CR} = \frac{f_{\text{ext}}}{f_{\text{int}}} \quad (5)
\]
were calculated by equation 1 using known CR and AUCI/AUC values.

 Wienholds et al., 1997

 Estimated values of the contribution ratio of CYP2C9 substrates

 TABLE 1

<table>
<thead>
<tr>
<th>Substrates</th>
<th>CR</th>
<th>f_m</th>
<th>References for f_m</th>
<th>AUC</th>
<th>References for AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>0.48</td>
<td>0.95</td>
<td>Yamazaki et al., 1998a; Tang et al., 1999</td>
<td>0.50</td>
<td>Sierfin and Fagele, 1979; Kumar et al., 2002</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>0.68</td>
<td>0.99</td>
<td>Tracy et al., 1996; Yamazaki et al., 1998a</td>
<td>0.69</td>
<td>Zgheib et al., 2007</td>
</tr>
<tr>
<td>Fluvoxastin</td>
<td>0.60</td>
<td>0.65</td>
<td>Andersson et al., 2004</td>
<td>0.92</td>
<td>To et al., 1992</td>
</tr>
<tr>
<td>S-Buprofen</td>
<td>0.50</td>
<td>0.70</td>
<td>Hamman et al., 1997</td>
<td>0.70</td>
<td>Rudy et al., 1991; Davies, 1998</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.50</td>
<td>0.60–0.80*</td>
<td>Chesné et al., 1998</td>
<td>0.70</td>
<td>Schmid et al., 1995</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>0.75</td>
<td>0.95</td>
<td>Miners et al., 1982</td>
<td>0.79</td>
<td>Dickinson et al., 1985</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>0.84</td>
<td>0.99</td>
<td>Miners and Birkett, 1998, 2000b</td>
<td>0.85</td>
<td>Madsen et al., 2001</td>
</tr>
<tr>
<td>S-Warfarin</td>
<td>0.69</td>
<td>0.96</td>
<td>Yamazaki et al., 1998a,b</td>
<td>0.72</td>
<td>Toon et al., 1986; Heimark et al., 1992</td>
</tr>
</tbody>
</table>

* Calculated by the extrapolation method (eq. 5).

 The contribution of CYP2C9 to hepatic clearance.

 The reference is only shown in Supplemental Material 3.

 These retrieved data were also used to predict in vivo DDIs by each of the in vitro-based methods. The steady-state concentrations [I] of the inhibitors were estimated in two ways: 1) total systemic C_max and 2) unbound hepatic inlet concentration (UHII) defined by eq. 6, where k_a is the absorption rate constant [0.03/min, an assumed average value (Obach et al., 2006)], F_m is the fraction absorbed (assumed to be 1), D is the dose of the inhibitor, Q_h is the liver blood flow (1498 ml/min), and f_u is the unbound fraction of drug in plasma. This equation assumes that metabolism of the inhibitor in the gut is negligible.

 \[ [I]_{\text{hepatic.inlet}} = f_u \cdot \left( \frac{C_{\text{max}} + F_m \cdot F_a \cdot D}{Q_h} \right) \] (6)

 For reversible inhibitors of CYP2C9 and CYP2D6, eq. 7 was used to predict the clinical DDI. The effect of multiple inhibitors was accounted for by summing the [I]/K_i ratios (Yeung et al., 2011). It should be pointed out that both f_mCYP (in vitro-based model) and CR (IVIP model) indicate the contribution ratio of the target metabolizing enzyme to the clearance of a substrate drug after oral absorption or intravenous administration, so the same value is used in our analysis.

 \[ \frac{AUC_1}{AUC} = \frac{1}{1 + \sum f_i \cdot \frac{[I]}{K_i}} + (1 - f_mCYP) \] (7)

 Predictions for reversible inhibitors of CYP3A used eq. 8 and incorporated the contribution of gut metabolism, where F_m is the fraction of absorbed substrate escaping gut metabolism by CYP3A. The concentration in the gut, [I]_gut, was defined by eq. 9, where Q_g is the enterocytic blood flow (248 ml/min).

 Predictions for reversible inhibitors of CYP3A used eq. 8 and incorporated the contribution of gut metabolism, where F_m is the fraction of absorbed substrate escaping gut metabolism by CYP3A. The concentration in the gut, [I]_gut, was defined by eq. 9, where Q_g is the enterocytic blood flow (248 ml/min).

 TABLE 2

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Inhibitors</th>
<th>Dose and Regimen</th>
<th>IR</th>
<th>CR</th>
<th>AUC/AUC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning set 1</td>
<td>Amiodarone</td>
<td>300, 3</td>
<td>0.31</td>
<td>0.69</td>
<td>1.27</td>
<td>Heimark et al., 1992</td>
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<tr>
<td>S-Warfarin</td>
<td>Benz bromaron</td>
<td>400, 3</td>
<td>0.76</td>
<td>0.69</td>
<td>2.11</td>
<td>O’Reilly et al., 1987</td>
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<td>S-Warfarin</td>
<td>Butoxolone</td>
<td>50, unknown</td>
<td>0.78</td>
<td>0.69</td>
<td>2.15</td>
<td>Takahashi et al., 1999b</td>
</tr>
<tr>
<td>S-Warfarin</td>
<td>Cimetidine</td>
<td>300, unknown</td>
<td>1.00</td>
<td>0.69</td>
<td>3.29</td>
<td>Tse et al., 1992</td>
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<tr>
<td>S-Warfarin</td>
<td>Diltiazem</td>
<td>1200, 3/15</td>
<td>0.33</td>
<td>0.69</td>
<td>1.30</td>
<td>Chu et al., 1998</td>
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<td>Tolbutamide</td>
<td>Diltiazem</td>
<td>60, 0</td>
<td>0.11</td>
<td>0.84</td>
<td>1.10</td>
<td>Dixit and Rao, 1999</td>
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<tr>
<td>S-Warfarin</td>
<td>Fluconazole</td>
<td>100, 7</td>
<td>0.38</td>
<td>0.84</td>
<td>1.35</td>
<td>Black et al., 1996</td>
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<tr>
<td>Tolbutamide</td>
<td>Fluconazole</td>
<td>200, 7</td>
<td>0.67</td>
<td>0.69</td>
<td>1.86</td>
<td>Neal et al., 2003</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>Fluvoxamine</td>
<td>300, 7</td>
<td>0.72</td>
<td>0.69</td>
<td>2.00</td>
<td>Neal et al., 2003</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>400, 7</td>
<td>0.94</td>
<td>0.69</td>
<td>2.84</td>
<td>Neal et al., 2003</td>
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<tr>
<td>Diclofenac</td>
<td>Fluvastatin</td>
<td>40, 7</td>
<td>0.42</td>
<td>0.48</td>
<td>1.25</td>
<td>Tronson et al., 1995</td>
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<td>Tolbutamide</td>
<td>Fluvastatin</td>
<td>40, 15</td>
<td>0.00</td>
<td>0.84</td>
<td>1.00</td>
<td>Appel et al., 1995</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>Fluvoxamine</td>
<td>75, 3</td>
<td>0.22</td>
<td>0.84</td>
<td>1.25</td>
<td>Madsen et al., 2001</td>
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<tr>
<td>Tolbutamide</td>
<td>150, 3</td>
<td>0.40</td>
<td>0.84</td>
<td>1.50</td>
<td>Madsen et al., 2001</td>
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<td>Tolbutamide</td>
<td>Ketokonazole</td>
<td>200, 7</td>
<td>0.52</td>
<td>0.84</td>
<td>1.77</td>
<td>Krishniah et al., 1994</td>
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<td>S-Warfarin</td>
<td>Miconazole</td>
<td>125, 3</td>
<td>1.00</td>
<td>0.69</td>
<td>4.72</td>
<td>O’Reilly et al., 1992</td>
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<tr>
<td>S-Warfarin</td>
<td>Paroxetine</td>
<td>30, 14</td>
<td>0.09</td>
<td>0.69</td>
<td>1.07</td>
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<td>Phenytoin</td>
<td>Sertaline</td>
<td>200, 17</td>
<td>0.21</td>
<td>0.75</td>
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<td>Tolbutamide</td>
<td>Sulfaphenazole</td>
<td>1000, 3/1</td>
<td>0.91</td>
<td>0.84</td>
<td>4.19</td>
<td>Back et al., 1988; Veronese et al., 1990</td>
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<tr>
<td>Tolbutamide</td>
<td>Sulfonpyrazone</td>
<td>800, 7</td>
<td>0.48</td>
<td>0.84</td>
<td>1.67</td>
<td>Miners et al., 1982</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Voriconazole</td>
<td>800, 9</td>
<td>0.60</td>
<td>0.75</td>
<td>1.81</td>
<td>Parkinson et al., 2003</td>
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<tr>
<td>Learning set 2</td>
<td>Losartan</td>
<td>200, 9/3</td>
<td>0.67</td>
<td>0.47</td>
<td>1.47</td>
<td>Kuzierad et al., 1997; Kaukonen et al., 1998</td>
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<td>Zafirlukast</td>
<td>Flunisal</td>
<td>200, 2*</td>
<td>0.67</td>
<td>0.56</td>
<td>1.60</td>
<td>Karonen et al., 2012</td>
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<td>Glimepiride</td>
<td>Fluconazole</td>
<td>200, 3</td>
<td>0.67</td>
<td>0.87</td>
<td>2.83</td>
<td>Nieminen et al., 2001</td>
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<td>Estraviron</td>
<td>Flunisal</td>
<td>200, 7</td>
<td>0.67</td>
<td>0.69</td>
<td>1.86</td>
<td>Kukada et al., 2011</td>
</tr>
</tbody>
</table>

# Milligrams per day, the daily doses of the inhibitors; days, the duration of the multiple dosing before administration of substrates. Single dose is shown as 0 days.

 The references are only shown in Supplemental Material 3.

 Only the DDIs associated with typical CYP2C9 substrates (S-warfarin, tolbutamide, diclofenac, and phenytoin) were included in the learning set 1. In learning set 1, IR of all CYP2C9 inhibitors were calculated by equation 1 using known CR and AUCI/AUC values.

 An algebraic mean of the AUCI/AUC was used if multiple studies are reported for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).

 Two IR values of fluvastatin were calculated and the mean value was used in the further analysis.

 In learning set 2, CR of the remaining CYP2C9 substrates (not included in Table 1) were calculated by eq. 3 using known IR and AUCI/AUC values.

 400 mg on the first day.
\[
\frac{AUC_I}{AUC} = \frac{1}{\left( \frac{f_{acyp}}{1 + \sum \left( \frac{[I]}{K_i} \right)} \right) + \left( 1 - f_{acyp} \right) + \frac{1}{1 + \left( \frac{[I]}{K_i} \right)}} \times \frac{1}{F_G + \frac{1 - F_G}{1 + \left( \frac{[I]}{K_i} \right)}}
\]

Predictions for irreversible inhibitors of CYP3A used eq. 10 with a \( k_{deg} \) (hepatic) of 0.000321/min (Obach et al., 2007). \( F_G \) of the substrate, and the degradation rate constant for CYP3A in the enterocty \( k_{deg} \) (gut) = 0.000481/min (Obach et al., 2007).

\[
\frac{AUC_I}{AUC} = \frac{1}{\left( \frac{f_{acyp}}{1 + \sum \left( \frac{[I]}{K_i} \right)} \right) + \left( 1 - f_{acyp} \right) + \frac{1}{1 + \left( \frac{[I]}{K_i} \right)}} \times \frac{1}{F_G + \frac{1 - F_G}{1 + \left( \frac{[I]}{K_i} \right)}}
\]

Assessment of Predictive Performance. To assess the quantitative accuracy of each model, a prediction error was calculated from the difference between each predicted AUC ratio and the observed ratio. The prediction bias of each assumption was calculated as an average deviation of the predicted versus observed AUC ratios. The precision of each assumption was calculated as an average deviation of the predicted between each predicted AUC ratio and the observed ratio. The prediction bias and specificity of each prediction model were standards for bioequivalence and weak inhibition (FDA Draft Guidance for decisions on the necessity for a clinical DDI study and approximates the FDA as “positive” if AUC ratio was ≥1.25 or otherwise were termed as “negative.” This threshold was selected to maximize our ability to make conservative decisions on the necessity for a clinical DDI study and approximates the FDA standards for bioequivalence and weak inhibition (FDA Draft Guidance for Industry, 2012). The sensitivity and specificity of each prediction model were determined. Sensitivity is a measure of the ability of the prediction approach to successfully identify a positive DDI. The specificity of a prediction approach is defined as its ability to successfully identify a negative (AUC ratio <1.25) or weak DDI (1.25 ≤ AUC ratio <2.00).

Results
Extending the IVIP Approach to CYP2C9-Mediated Interactions. A total of 44 different combinations (substrate and inhibitor) of in vivo DDI studies were identified by the literature search. The estimated values of CR for CYP2C9 substrates determined by the extrapolation method are listed in Table 1. Calculated IR values of all CYP2C9 inhibitors and CRs of the remaining four substrates (whose CR cannot be estimated by the extrapolation method) are shown in Table 2, which comprises learning sets 1 and 2.

References for the DDI studies involving CYP2C9 that were used for the external validation are shown in Table 3. A total of 19 AUC ratios were available. The relationship between the observed and predicted AUC ratios is plotted in Fig. 1A. All the points are inside the range of acceptable predictions (50–200%). The prediction accuracy of AUC/I/AUC ranged from 70 to 125% (Fig. 1B). The predictive sensitivity and specificity were both 93%. The predictive error and precision were −0.09 and 0.29, respectively.

The AUC/I/AUC ratios of 180 possible interactions between the 12 substrates and the 15 inhibitors listed in Tables 2 and 3 were calculated (Fig. 1C). Only a small proportion (21%) of all possible combinations between substrates and inhibitors had been studied in vivo.

Evaluation of the IVIP Approach in Predicting the DDI Risks of Various Inhibitors with Circulating Inhibitory Metabolites. The details of the data and calculation are provided as Supplemental Material 1. A total of 14 different combinations of in vivo DDI studies (including 12 inhibitors with inhibitory metabolites) were identified (Table 4). Two inhibitors (diltiazem and erythromycin) and their metabolites were shown to possess both reversible and irreversible inhibitory effects on CYP3A (Zhang et al., 2009a). However, both the reversible and irreversible in vitro models could not accurately predict the AUC ratios (Table 4). In general, the predictive performance of the model incorporating unbound UHI was not superior to the model that used total systemic \( C_{max} \) (Fig. 2). The predictive error of the three in vitro-based models was 6.62 (parent drug, \([I] = C_{max}\) ), 9.81 (parent drug and metabolites, \([I] = C_{max}\) ), and 1.77 (parent drug and metabolites, \([I] = UHI\) ), respectively. The precision of these in vitro-based models was 24.9, 35.4, and 8.06, respectively.

### TABLE 3

CYP2C9-mediated drug-drug interaction studies that were used for external validation

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Inhibitor</th>
<th>Dose and Regimen</th>
<th>IR</th>
<th>CR</th>
<th>Observed AUC/I/AUC</th>
<th>Predicted AUC/I/AUC</th>
<th>Accuracy</th>
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\* Milligrams per day, the daily doses of the inhibitors; days, the duration of the multiple dosing before administration of substrates.

\( The \) AUC/I/AUC \( \) was predicted by eq. 1.

\( The \) references are only shown in Supplemental Material 3.

\( 400 \) mg on the first day.

\( 800 \) mg on the first day.

An algebraic mean of the observed AUC/I/AUC was used if multiple studies are reported for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).
In contrast, the predictive error (0.10), precision (0.57), and absolute accuracy (75–128%) of the IVIP approach were significantly better. The predictive sensitivity of the in vitro model could be improved to some extent, when data pertaining to metabolites were included in the model (57% versus 79%). The IVIP approach could successfully identify 12 of 14 positive DDIs (86%). For the two failures, the observed versus predicted AUC ratios were 1.28 versus 1.17 and 1.59 versus 1.29, respectively. The predictive specificities of all three in vitro-based models and the IVIP approach were 86, 71, 86%, and 100%, respectively.

Discussion

There were two primary findings of this study. The first finding is that the modified IVIP approach can be extended to the prediction of CYP2C9-mediated DDIs. For the external validation set, the prediction accuracy for AUC ratios ranged from 70 to 125%. The second finding is that the accuracy of the IVIP approach in predicting DDI risks of 12 inhibitors with circulating inhibitory metabolites was more accurate than in vitro-based methods.

To our knowledge, this is the first proof-of-concept study demonstrating that the IVIP approach is a useful tool for the prediction of drug interaction risks associated with P450 inhibitors that have circulating inhibitory metabolites. The IR in IVIP model relies on in vivo data, thereby avoiding the confounding issues due to extrapolation from in vitro $K_i$ (or $k_{inact}/K_i$). The IVIP approach accommodates important confounding factors (inhibitory metabolites, different mechanisms of inhibition, and intestinal inhibition) in the prediction of DDIs, which can be difficult to handle by in vitro-based methods. The theoretical basis for the versatility of the IVIP approach is provided in Supplemental Material 2. In brief, the versatility of the IVIP approach is dependent on the IR of the inhibitors. The underlying meaning of the IR is different for different confounding factors. For instance, the IR reflects the total extent of inhibition occurring in the liver and intestines, if intestinal inhibition exists. In cases in which there are multiple inhibitors that act via independent inhibition mechanisms, the IR reflects the combined inhibitory effects of all inhibitors present.

Another advantage of the IVIP approach is that it is easy to use and does not require statistical or pharmacokinetic simulation software to perform the analysis.

Although the IVIP approach was developed on the basis of previous methods (Ohno et al., 2007; Tod et al., 2011), some differences merit discussion. In the current study, only the well-known CYP2C9 probes (S-warfarin, tolbutamide, diclofenac, and phenytoin) were eligible for the learning set used to calculate the IR values of various inhibitors. This criterion is likely to minimize the possibility of introducing misleading CR information. In addition, the IR values of the inhibitors that are calculated on the basis of reliable CYP2C9 probes will only reflect the inhibition of CYP2C9 rather than other inhibition mechanisms (such as transporter-mediated inhibition). In the current study, the CR values of most CYP2C9 substrates cannot be directly estimated from in vivo information because relevant data for CYP2C9 are limited. To overcome this difficulty, estimation of CR values was mainly based on the extrapolation method, using in vitro data. This modified approach was demonstrated to be accurate and reliable, suggesting that the extrapolation method is a reasonable alternative for estimation of CR values. The data from both oral and intravenous administration were included in our prediction of CYP2C9-mediated DDIs because the activity of CYP2C9 in the intestine is only 4% of the activity in the liver (Galetin and Houston, 2006).

The IVIP model for CYP2C9-mediated DDIs was also used to forecast the magnitude of a large number of drug interactions that have not been studied. The most potent CYP2C9 inhibitors are predicted to be bucolome and miconazole. Respectively, they caused 6.25- and 6.25-fold increases in the plasma AUC values of the DDI increased from yellow to red; the magnitude of the DDIs increased from 150% to 200% ranges of the prediction accuracy, prediction accuracy for the external validation set (B), and predicted AUC of substrates in the presence of various inhibitors (C). In C, the magnitude of the DDI increased from yellow to red; the doses for inhibitors amiodarone, fluconazole, and fluvoxamine are 400, 400, and 150 mg/day, respectively. The doses for other inhibitors are shown in Table 2. LOS, losartan; DIC, diclofenac; IBU, S-ibuprofen; MEL, meloxicam; ZAF, zafirlukast; FLT, fluvoxatim; FLX, flurbiprofen; ETR, etravirine; WAR, S-warfarin; PHE, phenytoin; TOI, tolbutamide; GLI, glimepiride; PAR, paroxetine; DIL, diltiazem; SER, sertraline; CIM, cimetidine; FLX, fluvoxamine; SUR, sulfanylpazone; KET, ketoconazole; VOR, voriconazole; AMI, amiodarone; BEN, benz bromarone; SUN, sulfaphenazole; FLN, fluconazole; BUC, buclo- lome; MIC, miconazole.

Fig. 1. Predicted versus observed AUC/AUC ratios (CYP2C9) in the external validation set (A; the dotted red line represents 50–200% ranges of the prediction accuracy), prediction accuracy for the external validation set (B), and predicted AUC of substrates in the presence of various inhibitors (C). In C, the magnitude of the DDI increased from yellow to red; the doses for inhibitors amiodarone, fluconazole, and fluvoxamine are 400, 400, and 150 mg/day, respectively. The doses for other inhibitors are shown in Table 2. LOS, losartan; DIC, diclofenac; IBU, S-ibuprofen; MEL, meloxicam; ZAF, zafirlukast; FLT, fluvoxatim; FLX, flurbiprofen; ETR, etravirine; WAR, S-warfarin; PHE, phenytoin; TOI, tolbutamide; GLI, glimepiride; PAR, paroxetine; DIL, diltiazem; SER, sertraline; CIM, cimetidine; FLX, fluvoxamine; SUR, sulfanylpazone; KET, ketoconazole; VOR, voriconazole; AMI, amiodarone; BEN, benz bromarone; SUN, sulfaphenazole; FLN, fluconazole; BUC, buclo- lome; MIC, miconazole.

Inhibitors IR/CR 0.47 0.48 0.50 0.50 0.56 0.60 0.68 0.69 0.69 0.75 0.84 0.87

Table 2. LOS, losartan; DIC, diclofenac; IBU, S-ibuprofen; MEL, meloxicam; ZAF, zafirlukast; FLT, fluvoxatim; FLX, flurbiprofen; ETR, etravirine; WAR, S-warfarin; PHE, phenytoin; TOI, tolbutamide; GLI, glimepiride; PAR, paroxetine; DIL, diltiazem; SER, sertraline; CIM, cimetidine; FLX, fluvoxamine; SUR, sulfanylpazone; KET, ketoconazole; VOR, voriconazole; AMI, amiodarone; BEN, benz bromarone; SUN, sulfaphenazole; FLN, fluconazole; BUC, buclo- lome; MIC, miconazole.
Inhibitors (Parent Drug) | Inhibitors (Metabolites) | Inhibitor Dose | Victim Drug | P450 | Observed AUC/Victor | Predicted AUC/Victor | References for Observed AUC/Victor
---|---|---|---|---|---|---|---
Casopitant | Hydroxycasopitant | 30 mg/kg | Midazolam | 3A | 1.76 | 1.41 | Zamuner et al., 2010
Diltiazem | N-DesmethylDiltiazem | 240 mg/kg | Midazolam | 3A | 3.88 | 1.41 | Backman et al., 1994; Zhang et al., 2009b
Erythromycin | N-Desmethyl erythromycin | 1500 mg/kg | Midazolam | 3A | 4.12 | 1.41 | Olikka et al., 1993; Zimmermann et al., 1996
Itraconazole | Hydroxyitraconazole | 200 mg/kg | Midazolam | 3A | 7.86 | 1.41 | Olikka et al., 1994; Backman et al., 1998
Itraconazole | Hydroxyitraconazole | 200 mg/kg | Simvastatin | 3A | 18.61 | 149.84 | Neuvonen et al., 1998
Bupropion | Erythrobupropion, hydroxybupropion, theobromobupropion | 150 mg/kg | Desipramine | 2D6 | 5.21 | 1.76 | Reese et al., 2008
Fluoxetine | Norfluoxetine | 20 mg/kg | Desipramine | 2D6 | 4.42 | 1.76 | Preskorn et al., 1994
Quinidine | Quinidine N-oxide, 3-hydroxyquinidine | 50 mg/kg | Desipramine | 2D6 | 3.14 | 1.76 | Ayesha et al., 1991
Sertraline | N-Desmethylsertraline | 50 mg/kg | Desipramine | 2D6 | 1.30 | 1.76 | Preskorn et al., 1994; Alderman et al., 1997
Sertraline | N-Desmethylsertraline | 150 mg/kg | Imipramine | 2D6 | 1.68 | 1.00 | Kurtz et al., 1997
Venlafaxine | N-Desmethylvenlafaxine | 150 mg/kg | Imipramine | 2D6 | 1.28 | 1.00 | Albers et al., 2000
Amiodarone | N-Desethylamiodarone | 200 mg/kg | Phenytoin | 2C9 | 1.40 | 1.36 | Nolan et al., 1989
Sulfipyrazole | Sulfipyrazole sulfide, Sulfipyrazole sulfone | 400 mg/kg | S-Warfarin | 2C9 | 1.82 | 2.24 | O’Reilly, 1982; Toon et al., 1986

a An algebraic mean of the observed AUC/Victor was used if multiple studies are reported for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).

b The data on the left were calculated based on the reversible inhibition model, whereas the data on the right were based on the irreversible inhibition model.

c Single dose of quinidine was administrated to the subjects in this study.

The moderate (amiodarone, benz bromarone, sulfaphenazole, and fluconazole) and weak (voriconazole, sulfipyrazole, and fluvoxamine) inhibitors of CYP2C9, our predictions are consistent with the FDA DDI guidance.

In the present study, the predictive performances of three in vitro-based models were compared with the IVIP approach. These in vitro data-based mathematical models require an assumption of the perpetrator concentration available to the enzyme ([I]/P). In this study, we suggest that different models should be used at the different stages of new drug development. Although the in vitro-based model is less accurate, it is useful in the earlier stages of drug development, before any clinical data are available (clinical [I]/P can be estimated based on preclinical data). If a new drug is a potential victim drug, one clinical DDI study for this victim drug (coadministered with a strong inhibitor) is needed to investigate the potential DDI risk (FDA Draft Guidance for Industry, 2012). The in vivo contribution ratio of this victim drug can be accurately estimated on the basis of the result of this clinical study. Then, the IVIP model can be used for predicting the DDI risks of this victim drug if it is coadministered with other inhibitors. According to the new draft guidance, when a strong inhibitor alters this victim drug, subsequent clinical studies or modeling is advised to define interactions with other less potent specific inhibitors. However, if no significant DDI is predicted for this victim drug and other weaker inhibitors based on the IVIP model, we suggest that a secondary clinical study may not be necessary. If a new drug is a potential perpetrator, most of the DDIs involving this drug can also be predicted using the IVIP model from a single clinical DDI study of the perpetrator and a sensitive substrate.
The limitations of the present study need to be considered. It is noteworthy that the value of IR is dose-dependent (as shown in Table 2) because it is estimated from specific in vivo study and the exposure of the perpetrator has already been considered with the certain relationship with $K_i$ or $K_{inact}/K_i$. Therefore, to accurately predict a certain clinical DDI, the dose and regimen of the perpetrator in the learning set should not be significantly different from that of the same perpetrator in this clinical DDI study. In addition, in its present form, the IVIP is not applicable to inhibitors that can inhibit both P450 enzymes and transporters such as P-glycoprotein. Failing to account for the interaction with P-glycoprotein may result in underprediction of the AUC ratio. However, our study included an inhibitor (quinidine) that inhibits both CYP2D6 and P-glycoprotein, but no significant underprediction of the AUC ratio was observed. This can be explained by the fact that both victim drugs (desipramine and metoprolol) in the validation set and learning set are not P-glycoprotein substrates. Further studies are underway in our laboratory to apply the model to transporter-mediated DDIs.

The IVIP approach is validated to be accurate in the prediction of CYP2C9-mediated DDIs and is a useful tool for the prediction of drug interaction risks associated with P450 inhibitors that have circulating inhibitory metabolites. This approach can be used in new drug development after the result of the first clinical DDI study is available.

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Authorship Contributions

**Participated in research design:** Hu, Parker, and Laizure.

**Conducted experiments:** Hu.

**Performed data analysis:** Hu.

**Wrote or contributed to the writing of the manuscript:** Hu, Parker, and Laizure.

**References**


FIG. 2. Predicted versus observed AUC/AUC ratios for in vivo drug-drug interaction studies associated with inhibitors that have inhibitory metabolites. The prediction was based on four different models. The dotted red lines represent 50 to 200% ranges of the prediction accuracy. For the inhibitors diltiazem and erythromycin, only the predicted AUC ratios based on the irreversible inhibition model were shown. In vitro (P), only the data of the parent drugs were included in the in vitro-based prediction approach and total systemic $C_{\text{max}}$ was used to estimate the perpetrator concentration available to the enzyme; in vitro (P+M), total systemic $C_{\text{max}}$ for the parent drug and metabolites were used in the in vitro-based prediction approach; in vitro UHI (P+M), unbound hepatic inlet concentrations of parent drug and metabolites were used to estimate the perpetrator concentration available to the enzyme.


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