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

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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 (28–805%). 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 According to the new FDA Draft Guidance for Industry (2012, 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.

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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; Einolf, 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_{mCYP}), 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; UHL, 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 ($0 \leq \text{CR} \leq 1$) and IR ($0 \leq \text{IR} \leq 1$) can be determined, then the ratio of the AUC in the presence (AUC_1) and absence (AUC) of the inhibitor can be estimated using eq. 1 (Ohno et al., 2007; Hisaka et al., 2010; Tod et al., 2011):

$$\frac{\text{AUC}_1}{\text{AUC}} = \frac{1}{1 - \text{CR} \cdot \text{IR}} \quad (1)$$

In contrast to the complex derivation by previous studies (Ohno et al., 2007; Tod et al., 2011), eq. 1 is readily derived from the well known *in vitro*-based model (reversible inhibition, intestinal metabolism ignored) below (eq. 2), where f_{mCYP} is the contribution of the specific enzyme to the overall clearance.

$$\frac{\text{AUC}_1}{\text{AUC}} = \frac{1}{\left(\frac{f_{\text{mCYP}}}{1 + \frac{[I]}{K_i}} \right) + (1 - f_{\text{mCYP}})} \quad (2)$$

After defining $f_{\text{mCYP}} = \text{CR}$ and $\text{IR} = [I]/([I] + K_i)$, eq. 2 is transformed to eq. 1. The IR in this model relies on *in vivo* data, thus avoiding the confounding issues due to extrapolation from K_i (or k_{inact}/K_i) and does not require distinction between reversible and irreversible (mechanism-based) inhibitors.

For the CYP2D6- and CYP3A-mediated DDIs, the CR values of most victim drugs can be estimated directly from *in vivo* data using the pharmacogenetic or drug interaction methods (Ohno et al., 2007; Tod et al., 2011). The

pharmacogenetic method (Tod et al., 2011) allows determination of CR from eq. 3, where AUC_{PM} is the AUC in poor metabolizers and AUC_{EM} is the AUC in extensive metabolizers.

$$\text{CR} = \frac{\text{AUC}_{\text{PM}}/\text{AUC}_{\text{EM}} - 1}{\text{AUC}_{\text{PM}}/\text{AUC}_{\text{EM}}} \quad (3)$$

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_1 is the AUC of the drug when the inhibitor is coadministered.

$$\text{CR} = \frac{\text{AUC}_1/\text{AUC} - 1}{\text{AUC}_1/\text{AUC} \cdot \text{IR}} \quad (4)$$

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_m is the contribution of CYP2C9 to hepatic clearance (estimated *in vitro* by CYP2C9-specific inhibitor or functional neutralizing antibody) and f_h 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).

$$\text{CR} = f_m \cdot f_h \quad (5)$$

The CR values of most CYP2C9 substrates were estimated by the extrapolation method ($\text{CR} = f_m \cdot f_h$) 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. 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.druginformation.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.

TABLE 1
Estimated values of the contribution ratio of CYP2C9 substrates

Substrates	CR ^a	f_m ^b	References for f_m ^d	f_h ^c	References for f_h ^d
Diclofenac	0.48	0.95	Yamazaki et al., 1998a; Tang et al., 1999	0.50	Stierlin and Faigle, 1979; Kumar et al., 2002
Flurbiprofen	0.68	0.99	Tracy et al., 1996; Yamazaki et al., 1998a	0.69	Zgheib et al., 2007
Fluvastatin	0.60	0.65	Andersson et al., 2004	0.92	Tse et al., 1992
S-Ibuprofen	0.50	0.70	Hamman et al., 1997	0.70	Rudy et al., 1991; Davies, 1998
Meloxicam	0.50	0.60–0.80 ^e	Chesné et al., 1998	0.70	Schmid et al., 1995
Phenytoin	0.75	0.95	Miners and Birkett, 1998; Komatsu et al., 2000b	0.79	Dickinson et al., 1985
Tolbutamide	0.84	0.99	Miners and Birkett, 1998; Komatsu et al., 2000a	0.85	Madsen et al., 2001
S-Warfarin	0.69	0.96	Yamazaki et al., 1998a,b	0.72	Toon et al., 1986; Heimark et al., 1992

^a Calculated by the extrapolation method (eq. 5).

^b The contribution of CYP2C9 to hepatic clearance.

^c The contribution of the hepatic clearance to the total clearance of the drug.

^d The references are only shown in Supplemental Material 3.

^e Value varies depending on the CYP3A4/CYP2C9 concentration ratio in different livers. A mean value was used in our analysis.

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 (UHI) defined by eq. 6, where k_a is the absorption rate constant [0.03/min, an assumed average value (Obach et al., 2006)], F_a 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.

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

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.

$$\frac{AUC_1}{AUC} = \frac{1}{\left(\frac{f_{mCYP}}{1 + \sum_j^n \left(\frac{[I]_j}{K_{ij}} \right)} \right) + (1 - f_{mCYP})} \quad (7)$$

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

TABLE 2

Data of learning set 1 (data selected to calculate the IR of all CYP2C9 inhibitors) and learning set 2 (data selected to calculate the CR of remaining CYP2C9 substrates)

Substrates	Inhibitors	Dose and Regimen ^a	IR	CR	AUC _i /AUC	References ^b
<i>mg/day, days</i>						
Learning set 1 ^c						
S-Warfarin	Amiodarone	300, 3	0.31	0.69	1.27	Heimark et al., 1992
		400, 3	0.76	0.69	2.11	O'Reilly et al., 1987
S-Warfarin	Benzbromarone	50, unknown	0.78	0.69	2.15	Takahashi et al., 1999b
S-Warfarin	Bucolome	300, unknown	1.00	0.69	3.29	Takahashi et al., 1999a
S-Warfarin	Cimetidine	1200, 3/15	0.33	0.69	1.30 ^d	O'Reilly, 1984; Sax et al., 1987
Tolbutamide	Diltiazem	60, 0	0.11	0.84	1.10	Dixit and Rao, 1999
S-Warfarin	Fluconazole	100, 7	0.38	0.69	1.35	Black et al., 1996
		200, 7	0.67	0.69	1.86	Neal et al., 2003
		300, 7	0.72	0.69	2.00	Neal et al., 2003
		400, 7	0.94	0.69	2.84	Neal et al., 2003
Diclofenac	Fluvastatin	40, 7	0.42 ^e	0.48	1.25	Transon et al., 1995
Tolbutamide	Fluvastatin	40, 15	0.00 ^e	0.84	1.00	Appel et al., 1995
Tolbutamide	Fluvoxamine	75, 3	0.22	0.84	1.23	Madsen et al., 2001
		150, 3	0.40	0.84	1.50	Madsen et al., 2001
Tolbutamide	Ketoconazole	200, 7	0.52	0.84	1.77	Krishnaiah et al., 1994
S-Warfarin	Miconazole	125, 3	1.00	0.69	4.72	O'Reilly et al., 1992
S-Warfarin	Paroxetine	30, 14	0.09	0.69	1.07	Bannister et al., 1989
Phenytoin	Sertraline	200, 17	0.21	0.75	1.19	Rapaport et al., 1996
Tolbutamide	Sulfaphenazole	1000, 3/1	0.91	0.84	4.19 ^d	Back et al., 1988; Veronese et al., 1990
Tolbutamide	Sulfapyrazole	800, 7	0.48	0.84	1.67	Miners et al., 1982
Phenytoin	Voriconazole	800, 9	0.60	0.75	1.81	Purkins et al., 2003
Learning set 2 ^f						
Losartan	Fluconazole	200, 9/3	0.67	0.47	1.47 ^d	Kazierad et al., 1997; Kaukonen et al., 1998
Zafirlukast	Fluconazole	200, 2 ^g	0.67	0.56	1.60	Karonen et al., 2012
Glimepiride	Fluconazole	200, 3 ^g	0.67	0.87	2.38	Niemi et al., 2001
Etravirine	Fluconazole	200, 7	0.67	0.69	1.86	Kakuda et al., 2011

^a 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.

^b The references are only shown in Supplemental Material 3.

^c 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 AUC_i/AUC values.

^d An algebraic mean of the 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).

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

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

^g 400 mg on the first day.

$$\frac{AUC_1}{AUC} = \frac{1}{\left(\frac{f_{mCYP}}{1 + \sum_j \left(\frac{[I]_j}{K_{ij}} \right)} \right) + (1 - f_{mCYP}) F_G + \left(\frac{1 - F_G}{1 + \frac{[I]_{gut}}{K_1}} \right)} \quad (8)$$

$$[I]_{gut} = \frac{k_a \cdot F_a \cdot D}{Q_g} \quad (9)$$

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 enterocyte [k_{deg} (gut) = 0.000481/min (Obach et al., 2007)].

$$\frac{AUC_1}{AUC} = \frac{1}{\left(\frac{f_{mCYP}}{1 + \sum_j \frac{[I]_j \cdot k_{inact,j}}{k_{deg} \cdot ([I]_j + K_{ij})}} \right) + (1 - f_{mCYP}) F_G + \left(\frac{1 - F_G}{1 + \frac{[I]_{gut} \cdot k_{inact}}{k_{deg(gut)} \cdot ([I]_{gut} + K_1)}} \right)} \quad (10)$$

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 the root mean square error. The observed and predicted DDIs were assigned 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 ($1.25 \leq$ 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_1/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_1/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 have been 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

Substrates	Inhibitor	Dose and Regimen ^a	IR	CR	Observed AUC ₁ /AUC	Predicted AUC ₁ /AUC ^b	Accuracy	References ^c
		mg/day, days					%	
Phenytoin	Amiodarone	200, 21	0.31	0.75	1.40	1.30	93	Nolan et al., 1989
Losartan	Bucolome	300, 7	1.00	0.47	1.67	1.90	114	Kobayashi et al., 2008
Tolbutamide	Cimetidine	1200, 3	0.33	0.84	1.20	1.39	116	Cate et al., 1986
Tolbutamide	Fluconazole	100, 6	0.38	0.84	2.09	1.47	70	Lazar and Wilner, 1990
Phenytoin	Fluconazole	200, 13	0.67	0.75	1.75	2.01	115	Blum et al., 1991
Fluvastatin	Fluconazole	200, 3 ^d	0.67	0.60	1.84	1.67	91	Kantola et al., 2000
Flurbiprofen	Fluconazole	200, 1 day/6days	0.67	0.68	1.92 ^e	1.84	96	Greenblatt et al., 2006; Kumar et al., 2008
		400, 6	0.94	0.68	3.03	2.77	91	Kumar et al., 2008
S-Ibuprofen	Fluconazole	200, 2 ^d	0.67	0.50	1.83	1.50	82	Hynninen et al., 2006
Losartan	Fluvastatin	40, 13	0.21	0.47	1.01	1.11	110	Meadowcroft et al., 1999
Glimepiride	Fluvoxamine	100, 3	0.22	0.87	1.33	1.24	93	Niemi et al., 2001
Etravirine	Paroxetine	20, 7	0.09	0.69	1.00	1.07	107	Brown et al., 2009
Tolbutamide	Sertraline	200, 21	0.21	0.84	1.19	1.22	102	Tremaine et al., 1997
Phenytoin	Sulfaphenazole	2000, 7	0.91	0.75	3.06	3.12	102	Hansen et al., 1979
S-Warfarin	Sulfapyrazone	400, 3	0.48	0.69	1.82 ^e	1.49	82	O'Reilly, 1982; Toon et al., 1986
Diclofenac	Voriconazole	400, 2 ^e	0.60	0.48	1.78	1.40	79	Hynninen et al., 2007
Meloxicam	Voriconazole	400, 2 ^e	0.60	0.50	1.46	1.43	98	Hynninen et al., 2009
Etravirine	Voriconazole	400, 7	0.60	0.69	1.36	1.70	125	Kakuda et al., 2011
S-Ibuprofen	Voriconazole	400, 2 ^e	0.60	0.50	2.01	1.43	71	Hynninen et al., 2006

^a Milligrams per day, the daily doses of the inhibitors; days, the duration of the multiple dosing before administration of substrates.

^b The AUC₁/AUC was predicted by eq. 1.

^c The references are only shown in Supplemental Material 3.

^d 400 mg on the first day.

^e 800 mg on the first day.

^f An algebraic mean of the observed AUC₁/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).

FIG. 1. Predicted versus observed AUC_r/AUC ratios (CYP2C9) in the external validation set (A); the dotted red lines represent 50–200% ranges of the prediction accuracy), prediction accuracy for the external validation set (B), and predicted AUC_r/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, fluvastatin; FLP, flurbiprofen; ETR, etravirine; WAR, *S*-warfarin; PHE, phenytoin; TOL, tolbutamide; GLI, glimepiride; PAR, paroxetine; DIL, diltiazem; SER, sertraline; CIM, cimetidine; FLX, fluvoxamine; SUR, sulfapyrazole; KET, ketoconazole; VOR, voriconazole; AMI, amiodarone; BEN, benzbromarone; SUN, sulfaphenazole; FLN, fluconazole; BUC, bucolome; MIC, miconazole.

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 1.24, 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 are 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 inhibitory 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 CYP2C9 probe tolbutamide (Fig. 1C). According to the FDA classifications of strong, moderate, or weak inhibitors (FDA Draft Guidance for Industry, 2012), these perpetrators may be strong inhibitors of CYP2C9. However, no strong inhibitor of CYP2C9 was listed in the new FDA draft guidance and a recent criteria-based assessment of perpetrators (Polasek et al., 2011). Although these three perpetrators have limited clinical application, they may serve as useful tools for drug metabolism studies. With regard to

TABLE 4

Predicted versus observed AUC ratios (AUC_i/AUC) calculated with the in vitro-based model and in vivo information-guided prediction approach

In vitro (P), only the data of the parent drugs were included in the in vitro-based prediction approach and total systemic $C_{\max}$ was used to estimate perpetrator concentration available to the enzyme; in vitro (P + M), both the data of the parent drug and metabolites were included in the in vitro-based prediction approach; in vitro UHI (P + M), unbound hepatic inlet concentration was used to estimate perpetrator concentration available to the enzyme.

Inhibitors (Parent Drug)	Inhibitors (Metabolites)	Inhibitor Dose	Victim Drug	P450	Observed AUC _i /AUC	Predicted AUC _i /AUC				References for Observed AUC _i /AUC
						In Vitro (P)	In Vitro (P + M)	In Vitro UHI (P + M)	IVIP	
		mg/kg								
Casopitant	Hydroxycasopitant	30	Midazolam	3A	1.76	1.36	1.41	1.31	1.74	Zamuner et al., 2010
Diltiazem	<i>N</i> -Desmethyldiltiazem	240	Midazolam	3A	3.88 ^a	1.38/15.99 ^b	1.56/20.73 ^b	1.59/22.98 ^b	3.91	Backman et al., 1994; Zhang et al., 2009b
Erythromycin	<i>N</i> -Desmethyl erythromycin	1500	Midazolam	3A	4.12 ^a	1.57/22.23 ^b	1.57/23.41 ^b	1.63/23.09 ^b	4.72	Olkkola et al., 1993; Zimmermann et al., 1996
Itraconazole	Hydroxyitraconazole	200	Midazolam	3A	7.86 ^a	19.52	21.34	13.61	8.93	Olkkola et al., 1994, 1996; Backman et al., 1998
Itraconazole	Hydroxyitraconazole	200	Simvastatin	3A	18.61	110.66	149.84	47.38	18.26	Neuvonen et al., 1998
Ranolazine	<i>O</i> -Desmethylanolazine	2000	Simvastatin	3A	1.59	2.72	2.79	3.66	1.24	Jerling et al., 2005
Bupropion	Erythrohydrobupropion, hydroxybupropion, threoerythrohydrobupropion	150	Desipramine	2D6	5.21	1.03	1.76	1.46	6.67	Reese et al., 2008
Fluoxetine	Norfluoxetine	20	Desipramine	2D6	4.42	2.09	3.29	1.47	4.67	Preskorn et al., 1994
Quinidine	Quinidine <i>N</i> -oxide, 3-hydroxyquinidine	50 ^c	Desipramine	2D6	3.14	5.27	5.29	5.64	2.64	Ayesh et al., 1991
Sertraline	<i>N</i> -Desmethylsertraline	50	Desipramine	2D6	1.30 ^a	1.00	1.01	1.00	1.54	Preskorn et al., 1994; Alderman et al., 1997
Sertraline	<i>N</i> -Desmethylsertraline	150	Imipramine	2D6	1.68	1.00	1.01	1.00	1.26	Kurtz et al., 1997
Venlafaxine	<i>N</i> -Desmethylvenlafaxine	150	Imipramine	2D6	1.28	1.00	1.00	1.12	1.17	Albers et al., 2000
Amiodarone	<i>N</i> -Desethylamiodarone	200	Phenytoin	2C9	1.40	1.01	1.36	1.02	1.30	Nolan et al., 1989
Sulfinpyrazone	Sulfinpyrazone sulfide, Sulfinpyrazone sulfone	400	<i>S</i> -Warfarin	2C9	1.82 ^a	1.16	2.24	1.01	1.49	O'Reilly, 1982; Toon et al., 1986

^a 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).

^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 administered to the subjects in this study.

the moderate (amiodarone, benzobromarone, sulfaphenazole, and fluconazole) and weak (voriconazole, sulfinpyrazone, 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]) for the prediction of DDIs. This elusive value is not easy to determine for human P450 drug metabolism. Therefore, alternative assumptions have been explored to optimize a static value for [I], including systemic $C_{\max}$, C_{avg} , and estimated hepatic inlet concentrations. However, at present there is no consensus on the in vivo inhibitor concentration that should be used. For instance, the FDA DDI guidance advocates that a clinical DDI study be conducted if the $[I]/K_i$ ratio >0.1 , where [I] = total systemic $C_{\max}$. It has also been reported that different estimates work better for different classes of inhibitors; for example, unbound systemic concentration has been used for predicting DDIs caused by irreversible inhibition, whereas the estimated unbound hepatic inlet concentration has been the preferred value for reversible inhibition (Obach, 2009). In a recent study, Shardlow et al. (2011) observed that unbound hepatic inlet concentration allowed the accurate prediction of DDIs for the drugs in their data set (including different types of inhibitors). Therefore, both total systemic $C_{\max}$ and estimates of unbound hepatic inlet concentrations were used as surrogates for the inhibitor concentration in our analysis. Furthermore, in vitro-based models differentiated reversible and irreversible inhibition and also considered the contribution of intestinal metabolism for CYP3A substrates.

Physiologically based pharmacokinetic approaches to define dynamic perpetrator and victim concentrations are becoming more widespread (Zhao et al., 2011). However, accurate prediction by this complex model relies on the accurate estimation of comprehensive in vivo and in vitro PK parameters of the substrates and inhibitors, as well as all of the inhibitory metabolites (Zhang et al., 2009b). These data are often not available for compounds early in drug discovery.

To improve decision making in drug development and discovery, 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] 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 physiologically based pharmacokinetic model may be used in the later stages of drug develop-

FIG. 2. Predicted versus observed AUC_i/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_{max} was used to estimate the perpetrator concentration available to the enzyme; in vitro (P+M), total systemic C_{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.

ment after more comprehensive clinical PK data and DDI data are available.

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.

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