ABSTRACT:

Attempts at predicting drug-drug interactions perpetrated by paroxetine from in vitro data have utilized reversible enzyme inhibition models and have been unsuccessful to date, grossly underpredicting interaction magnitude. Recent data have provided evidence for mechanism-based inactivation of CYP2D6 by paroxetine. We have predicted the pharmacokinetic consequences of CYP2D6 inactivation by paroxetine from in vitro inhibition kinetics ($K_{inact}$ 0.17 min$^{-1}$, unbound $K_I$ 0.315 μM), in vivo inhibitor concentrations, and an estimated CYP2D6 degradation half-life of 51 h, using a mathematical model of mechanism-based inhibition. The model-predicted accumulation ratio of paroxetine was 5 times that expected from single-dose kinetics and in excellent agreement with the observed 5- to 6-fold greater accumulation. Magnitudes of interactions produced by paroxetine (20–30 mg/day) with desipramine, risperidone, perphenazine, atomoxetine, (S)-metoprolol, and (R)-metoprolol were predicted, considering the contribution of CYP2D6 to their oral clearance. Predicted fold-increases in victim drug AUC were 5-, 6-, 5-, 6-, 4-, and 6-fold, respectively, and are in reasonable agreement with observed values of 5-, 6-, >7-, 7-, 5-, and 8-fold, respectively. Failure to consider microsomal binding in vitro adversely affected predictive accuracy. Simulation of the sensitivities of these predictions to model inputs suggests a 2-fold underprediction of interaction magnitude when a CYP2D6 degradation half-life of 14 h (reported for rat CYP3A) is used. In summary, the scaling model for mechanism-based inactivation successfully predicted the pharmacokinetic consequences of CYP2D6 inactivation by paroxetine from in vitro data.

INTRODUCTION

Paroxetine (Paxil) is a widely used selective serotonin reuptake inhibitor antidepressant that is indicated for the treatment of major depressive disorder, obsessive compulsive disorder, panic disorder, generalized anxiety disorder, social anxiety disorder, and post-traumatic stress disorder.

Paroxetine is an established perpetrator of drug-drug interactions (DDIs) when coadministered with agents whose clearance is largely dependent on the activity of cytochrome P450 2D6 (CYP2D6). Examples of characterized clinical interactions with paroxetine include its effects on the kinetics of desipramine (Børsen et al., 1993; Alderman et al., 1997), perphenazine (Ozdemir et al., 1997), metoprolol (Hemeryck et al., 2000), risperidone (Spina et al., 2001), and atomoxetine (Belle et al., 2002), where the clearance of the victim drugs is impaired by 5- to 8-fold. In addition, paroxetine displays nonlinear accumulation kinetics with steady-state exposures exceeding projections from single-dose kinetics by ~5-fold in CYP2D6 extensive metabolizers but not in CYP2D6 poor metabolizers (Kaye et al., 1989; Sindrup et al., 1992). This finding has been attributed to metabolic saturation following multiple dosing (Sindrup et al., 1992), although supporting evidence at the enzyme kinetic level is lacking. In vitro studies have reproducibly demonstrated potent inhibition of human liver microsomal CYP2D6 activity via an apparent competitive mechanism (von Moltke et al., 1995; Otton et al., 1996; Hemeryck et al., 2001). However, attempts at in vitro-in vivo extrapolation (IVIVE) of interaction magnitude under the assumption of reversible inhibition have been largely unsuccessful, even when nonspecific microsomal binding in vitro was considered (Hemeryck et al., 2001) or empirical approaches such as application of total plasma or even total intrahepatic concentrations of paroxetine were used in the predictions (von Moltke et al., 1995; Hemeryck et al., 2000).

Recent data have provided evidence for mechanism-based inactivation (MBI) of CYP2D6 by paroxetine (Bertelsen et al., 2003). Paroxetine produced a concentration- and time-dependent inhibition of human liver microsomal CYP2D6 activity in vitro, as measured by dextromethorphan O-demethylation rate. Kinetic analysis revealed that paroxetine produced a metabolism-dependent rapid loss of activity of the enzyme with a half-life of inactivation of 4 min ($k_{inact}$ 0.17 min$^{-1}$), and biochemical evidence for metabolite-intermediate complexation via a carbene-heme complex with CYP2D6 (Bertelsen et al., 2003).

Whereas in vitro-in vivo scaling approaches for reversible inhibition of P450 enzymes have been extensively described over the last decade and applied in the prediction of drug-drug interactions, mathematical models for scaling interactions resulting from mechanism-based inhibition have only recently been described, with relatively
fewer reported examples illustrating their predictive utility. Of notable mention are the reasonably successful predictions of the interactions perpetrated by fluoxetine, diltiazem, clarithromycin, verapamil, and the HIV protease inhibitors with CYP3A4 substrates using a mathematical model of mechanism-based inhibition developed by Hall and colleagues (Mayhew et al., 2000; Wang et al., 2004; Ernest et al., 2005). In vitro-in vivo extrapolations using this model require a formal kinetic analysis of inactivation by the perpetrator agent to determine the potency of inactivation (Ki) and maximal inactivation rate (kdegrad). An estimate of the enzyme-available concentration of the inhibitor is necessary (as for scaling models for reversible inhibition). In addition, an estimate of the in vivo degradation rate (kdegrad) of the enzyme in humans is needed.

In this report, we describe the application of the scaling model for mechanism-based inhibition in predicting the pharmacokinetic consequences of CYP2D6 inactivation by paroxetine from in vitro data. Specifically, we describe predictions of 1) the nonlinear accumulation kinetics of paroxetine resulting from autoinhibition of clearance; and 2) the magnitude of drug-drug interactions of paroxetine with CYP2D6 substrate victim drugs. In addition, we have characterized the sensitivity of these predictions to estimates of model inputs that are nearly impossible to determine directly in humans and, therefore, are associated with the greatest uncertainty: in vivo enzyme degradation rate and the in vivo enzyme-available inhibitor concentration. The implications of these findings for assessment of clinical risk associated with mechanism-based inhibitory DDIs are discussed.

Materials and Methods

Scaling Model. The following mathematical model has been previously derived for IVIVE of drug interactions resulting from MBI (Mayhew et al., 2000):

\[
\frac{CL_{\text{int},1}}{CL_{\text{int},\text{control}}} = \frac{k_{\text{degrad}}}{f_{\text{I}} \times k_{\text{max}}} + \frac{k_{\text{degrad}}}{f_{\text{I}} + K_i}
\]  

(1)

In eq. 1, CL_{\text{int},1} and CL_{\text{int},\text{control}} are the intrinsic clearance of the metabolic pathway of the victim drug in the presence and absence of the mechanism-based inactivator (concentration I). The inactivation rate constant k_{\text{max}} (maximal inactivation rate at saturating concentration of the inactivator) and the potency K_i (inactivator concentration at which half-maximal inactivation rate is achieved), which are typically measured in vitro, are the primary kinetic parameters describing the MBI process, and k_{\text{degrad}} represents the first-order rate constant for degradation (turnover) of the enzyme in vivo. If the victim drug is dosed orally, completely absorbed, and cleared entirely by hepatic processes: CYP2D6 turnover half-life and paroxetine steady-state half-life [18.3 h after dosing at 20 mg/day (Kaye et al., 1989)]. The CYP2D6 turnover half-life was thus determined using a noncompartmental deconvolution (component analysis) approach. Deconvolution of the mean residence time of paroxetine at steady state of 26.4 h (18.3/0.693) afforded a mean CYP2D6 degradation time of 74 h (100.4/26.4), translating to a CYP2D6 degradation half-life of 51 h (0.693 × 74) and a first-order degradation rate constant k_{\text{degrad}} of 0.0136 h⁻¹ for CYP2D6. The inhibitor concentration (I) was estimated as the unbound steady-state average concentration of paroxetine from total systemic exposures that were either measured in the specific drug-drug interaction studies or reported in previously published literature on the clinical pharmacokinetics of paroxetine (Kaye et al., 1989), and an unbound fraction (f_a) of 0.05 in human plasma (Kaye et al., 1999). Other estimates of inhibitor concentration including the total systemic concentration and the steady-state maximum unbound concentration at the inlet to the liver (estimated using eq. 5, which includes the steady-state systemic paroxetine C_{\text{max}} and an additive portal venous “absorption” component as described by Kanamitsu et al., 2000) were also evaluated as alternative inputs to the model.

\[
I_{\text{inact,max}} = f_a \times I_{\text{inact}} = f_a \times \left( C_{\text{max}} + \frac{k_{\text{I}} \times Dose \times f_a}{Q_h} \right)
\]  

(5)

For application of eq. 5, the absorption rate constant (k_a) for paroxetine was estimated as 0.01 min⁻¹ using eq. 6, based on a mean T_{\text{max}} of 5 h and a half-life of 23 h (elimination rate constant k_{el} of 0.03 h⁻¹). Complete absorption (f_a = 1) of paroxetine was assumed based on the human absorption/disposition of paroxetine (Kaye et al., 1989), and a hepatic blood flow (Q_h) of 1500 ml/min was assumed (Davies and Morris, 1993).

\[
T_{\text{max}} = \ln \left( \frac{k_a}{k_{\text{el}}} \right)
\]  

(6)

The fractional contribution of CYP2D6 to the overall clearance of each victim drug (f_{2D6}) was estimated from its clinical pharmacokinetics in CYP2D6 extensive metabolizers and poor metabolizers (Table 1) as follows, where CL_{\text{CYP2D6}} is the clearance of CYP2D6 (PM) or CYP2D6 (EM) (or their average concentration value when the respective apparent oral clearance...
values (or area under the plasma concentration-time curve following an oral dose) of the victim drug [atomoxetine (Sauer et al., 2003), desipramine (Brosen et al., 1993), (R)-metoprolol (Lennard et al., 1983), (S)-metoprolol (Lennard et al., 1983), paroxetine (Sindrup et al., 1992), perphenazine (Dahl-Puustinen et al., 1989), (-)-metoprolol (Lennard et al., 1983), (R)-metoprolol (Lennard et al., 1983), (R)-dopamine (Huang et al., 1993) in the poor metabolizer and extensive metabolizer subpopulations:

\[ f_{\text{EM}} = \left( \frac{1}{1 + K_I \cdot C} \right) \]

**Sensitivity Analysis.** The sensitivities of the model-derived predictions of drug interaction magnitude (fold-increase in victim drug AUC) to uncertainties in estimates of CYP2D6 degradation half-life and enzyme-available inhibitor concentration \( (I) \) were simulated over a wide range of relevant values for these input parameters (CYP2D6 half-life values of 10–100 h, and enzyme-available paroxetine concentrations of 1–1000 nM) for victim drugs, with varying relative contributions of CYP2D6 to overall oral clearance and enzyme-available inhibitor concentrations. The results of these simulations are shown in Table 1.

### Results

**Prediction of Nonstationary Pharmacokinetics of Paroxetine.** Steady-state average plasma concentrations of 35 to 42.5 ng/ml are achieved following dosing with paroxetine at 20 to 30 mg q.d., translating to unbound systemic exposures of 5.3 to 6.5 nM (Kaye et al., 1989; Sindrup et al., 1992). Application of the scaling model with the previously described input parameters \( (k_{\text{inact}}, K_I) \) suggests that in patients receiving paroxetine at 20 to 30 mg q.d. doses, 95% of CYP2D6 will be inactivated. Upon consideration of paroxetine’s \( f_{\text{EM}} \) of 0.86, the net decrement in paroxetine oral clearance at steady state as a consequence of autoinactivation of CYP2D6-mediated hepatic metabolism is predicted to be 80%, translating to a predicted accumulation ratio that is 5 times that expected if the kinetics were time-independent. This prediction is in excellent agreement with the observed 5.9- to 5.7-fold greater than expected accumulation following multiple dose administration of paroxetine at 20 to 30 mg q.d. in clinical pharmacokinetic studies (Kaye et al., 1989; Sindrup et al., 1992).

However, when nonspecific microsomal binding of paroxetine in vitro was not considered in the scaling process (that is, a “total” \( K_I \) value of 4.85 \( \mu M \) was used as input to the model), the predicted percentage loss of CYP2D6 activity following multiple dose administration of paroxetine at 20 to 30 mg q.d. was only 45 to 50%, translating to an approximately 40% decrement in paroxetine steady-state oral clearance, and accumulation that is 1.7-fold in excess of that expected from the single-dose kinetic profile. Failure to consider nonspecific microsomal binding in vitro therefore resulted in an approximately 3-fold underprediction of the extent of nonlinearity in accumulation of paroxetine.

Application of total systemic steady-state average concentrations of paroxetine [106–130 nM following dosing with paroxetine at 20 to 30 mg q.d. (Kaye et al., 1989; Sindrup et al., 1992)] or estimated steady-state maximum unbound concentrations of paroxetine at the inlet to the liver (\( \sim 27–40 \text{nM following dosing with paroxetine at 20 to 30 mg q.d.} \), calculated using eq. 5 as inputs to the scaling model, did not increase predictive accuracy (a 6- to 7-fold greater than expected accumulation was predicted, compared with a 5-fold prediction using steady-state average systemic unbound concentrations).

The performance of the scaling model in predicting the steady-state kinetics of paroxetine from the single-dose kinetic profile is illustrated in Fig. 1. For purposes of this exercise, previously published data on the single-dose kinetics of paroxetine (Sindrup et al., 1992) were fitted to a one-compartment pharmacokinetic model, and the following parameters were estimated: first-order absorption rate constant \( (k_{\text{a}}) \), 0.908 h\(^{-1}\); apparent volume of distribution \( (V/F) \), 3087 liters; and elimination rate constant \( (k_{\text{el}}) \), 0.063 h\(^{-1}\). The expected steady-state profile following administration of 14 daily doses of 30 mg was traced by superposition of the fitted single-dose kinetic profile (WinNonlin Enterprise version 3.2 software; Pharsight, Mountain View, CA),
assuming linear accumulation kinetics. These profiles for the 1st and 14th doses are represented in Fig. 1 by solid lines. Assuming a hepatic blood flow of 21 ml/min/kg (Davies and Morris, 1993), the impact of autoinactivation on the systemic clearance and oral bioavailability of paroxetine at steady state were estimated from the model-predicted fractional decrement in apparent oral clearance (intrinsic clearance), in the context of a well stirred pharmacokinetic model (Wilkinson and Shand, 1975). Assuming that autoinactivation does not alter volume of distribution [but would alter the apparent volume of distribution (Vd/F) due to alteration of the bioavailability, F] or absorption kinetics, and that changes in systemic clearance will be reflected as changes in elimination half-life, the following pharmacokinetic parameters for paroxetine post-CYP2D6 inactivation were derived: apparent volume of distribution (Vd/F), 1397 liters; and elimination rate constant (ke), 0.0282 h⁻¹. The corresponding superpositioned steady-state profile is represented in Fig. 1 by a dashed line profile and is in reasonably good agreement with the observed steady-state pharmacokinetic data. In contrast, when nonspecific microsomal binding is not considered in the scaling, the resulting pharmacokinetic parameter estimates [apparent volume of distribution (Vd/F) 2262 liters; and elimination rate constant (ke) 0.0525 h⁻¹] predict a steady-state profile (represented by the dotted line) that substantially underpredicts the observed extent of accumulation.

**Prediction of Drug Interaction Magnitudes.** The results of in vitro-in vivo extrapolation of drug interaction magnitude for CYP2D6 substrate victim drugs upon coadministration of paroxetine are detailed in Table 2. The scaling model predicts that administration of 20 to 30 mg daily doses of paroxetine results in a >90% loss of CYP2D6 activity (FDCL2D6 0.9–0.95). Across all victim drugs and drug interaction studies, the mean accuracy was 117% (range, 90–140%) when the total systemic concentration was used, and was 127% (range, 97–140%) when the total systemic concentration was used.

The performance of the scaling model is illustrated in Fig. 2 for the interaction of paroxetine with desipramine (Alderman et al., 1997). For purposes of this exercise, the steady-state pharmacokinetic profile of desipramine (50 mg q.d.) in the control phase (Alderman et al., 1997) was fitted to a one-compartment pharmacokinetic model with an absorption lag time and the following parameters estimated: first-order absorption rate constant (ka), 0.381 h⁻¹; absorption lag time (tlag), 0.79 h; apparent volume of distribution (Vd/F) 1762 liters; and elimination rate constant (ke), 0.0408 h⁻¹. Assuming a hepatic blood flow of 21 ml/min/kg (Davies and Morris, 1993), the impact of CYP2D6 inactivation by paroxetine on the systemic clearance and oral bioavailability of desipramine were estimated from the model-predicted fractional decrement in apparent oral clearance (FDCLtot 0.81 at a paroxetine dosage of 30 mg q.d.; Table 2), in the context of a well stirred pharmacokinetic model (Wilkinson and Shand, 1975). Assuming that the interaction does not alter volume of distribution [but would alter the apparent volume of distribution (Vd/F) due to alteration of F] or absorption kinetics, and that changes in systemic clearance will be reflected as changes in desipramine’s elimination half-life, the following pharmacokinetic parameters for desipramine post-CYP2D6 inactivation were derived: apparent volume of distribution (Vd/F), 1122 liters; and elimination rate constant (ke), 0.0122 h⁻¹. The corresponding superpositioned steady-state profile is represented in Fig. 2 by a dashed line profile and is in reasonably good agreement with the observed steady-state pharmacokinetic profile (Alderman et al., 1997) of desipramine (50 mg q.d.) with coadministered paroxetine (30 mg q.d. to steady state). In contrast, when nonspecific microsomal binding is not considered in the scaling (FDCLtot 0.473 at a paroxetine dosage of 30 mg q.d.; Table 2), the resulting pharmacokinetic parameter estimates [apparent volume of distribution (Vd/F), 1387 liters; and elimination rate constant (ke), 0.0273 h⁻¹] predict a steady-state profile (represented by the dotted line in Fig. 2) that substantially underpredicts the observed interaction, illustrating the importance of nonspecific microsomal binding considerations in quantification of inactivator potency.

**Sensitivity Analyses.** Simulations of the sensitivity of the model-predicted fold-increase in victim drug AUC to the estimated enzyme-available inhibitor concentration and CYP2D6 degradation half-life are shown in Figs. 3 and 4, respectively. Sensitivities of the predicted interaction magnitude to both these input parameters were greatest when the victim drug was exclusively cleared by CYP2D6 (f2D6 ≈ 1.0). For the victim drugs investigated here (f2D6 ≤ 0.9), use of steady-state average total plasma paroxetine concentration following

<table>
<thead>
<tr>
<th>Victim Drug</th>
<th>f2D6</th>
<th>PX</th>
<th>[PX]</th>
<th>Predicted FDCL2D6 Microsomal binding considered?</th>
<th>Predicted FDCLtot Microsomal binding considered?</th>
<th>Predicted AUC Ratio Microsomal binding considered?</th>
<th>Actual AUC Ratio</th>
<th>Percentage Accuracy Microsomal binding considered?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/day</td>
<td>Nm</td>
<td></td>
<td>Predicted FDCL2D6 Microsomal binding considered?</td>
<td>Predicted FDCLtot Microsomal binding considered?</td>
<td>Predicted AUC Ratio Microsomal binding considered?</td>
<td>Actual AUC Ratio</td>
<td>Percentage Accuracy Microsomal binding considered?</td>
</tr>
<tr>
<td>Atomoxetine</td>
<td>0.90</td>
<td>20</td>
<td>4.6a</td>
<td>0.413 0.915</td>
<td>0.374 0.827</td>
<td>1.6 5.8</td>
<td>7.1</td>
<td>23 82</td>
</tr>
<tr>
<td>Desipramine</td>
<td>0.85</td>
<td>20</td>
<td>5.0b</td>
<td>0.437 0.922</td>
<td>0.373 0.786</td>
<td>1.6 4.7</td>
<td>5.2</td>
<td>31 90</td>
</tr>
<tr>
<td>(R)-Metoprolol</td>
<td>0.89</td>
<td>20</td>
<td>5.9</td>
<td>0.478 0.933</td>
<td>0.427 0.833</td>
<td>1.7 6.0</td>
<td>7.9</td>
<td>22 76</td>
</tr>
<tr>
<td>(S)-Metoprolol</td>
<td>0.80</td>
<td>20</td>
<td>5.9</td>
<td>0.478 0.933</td>
<td>0.383 0.748</td>
<td>1.6 4.0</td>
<td>5.1</td>
<td>31 78</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>0.87</td>
<td>20</td>
<td>5.9</td>
<td>0.478 0.933</td>
<td>0.416 0.811</td>
<td>1.7 5.3 &gt;7.0f</td>
<td>N.C.≥</td>
<td>N.C.</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.89</td>
<td>20</td>
<td>5.9</td>
<td>0.478 0.933</td>
<td>0.428 0.825</td>
<td>1.7 5.7</td>
<td>5.5</td>
<td>31 104</td>
</tr>
</tbody>
</table>

a Paroxetine steady-state plasma unbound concentration from the exposure measured in this study.

b Paroxetine steady-state plasma unbound concentration following 20 mg q.d. dosing (Kaye et al., 1989).

c The 7-fold increase is a lower bound, since half-life was prolonged but only AUC0-8 h was measured; predicted accuracy is thus not calculated (N.C.) for the perphenazine-paroxetine interaction.

Table 2: Model-predicted magnitudes of drug-drug interactions of paroxetine with CYP2D6 substrates, without and with consideration of nonspecific microsomal binding

FDCL2D6 and FDCLtot refer to the fractional decrement in oral clearance via the CYP2D6 component of net clearance and the fractional decrement in total oral clearance, respectively; f2D6 refers to the fractional contribution of CYP2D6 to net apparent oral clearance; and [PX] is the steady-state average plasma unbound concentration of paroxetine (PX).
10 mg b.i.d. or 20 to 30 mg q.d. dosing in the various reported paroxetine DDI studies (\(I_{ss}\) in the 80–160 nM range) or corresponding estimates of steady-state maximum portal venous unbound concentration \(I_{inlet,max,u}\) 17–42 nM using eq. 5 instead of steady-state average unbound concentration \(I_{ss,u}\) in the 4–8 nM range) did not significantly alter the predicted magnitude of the drug interaction,
since this range of inhibitor exposures (4–160 nM) is outside the
dynamic range of the sensitivity curve (Fig. 3). Figure 4 suggests that
the use of a 14-h half-life for CYP2D6 [as used for CYP3A4 (May-
hew et al., 2000), based on the half-life of rat CYP3A] instead of the
51-h estimate of human CYP2D6 half-life would result in a 2-fold
underprediction of interaction magnitude.

Discussion

The predictive utility of a previously described mathematical model
for IVIVE of DDIs resulting from MBI (Mayhew et al., 2000) has
been demonstrated in this investigation for CYP2D6-inhibitory DDIs
involving the selective serotonin reuptake inhibitor antidepressant
paroxetine. Across substrates, the accuracy of the model in predicting
the magnitude of DDIs was approximately 90%. Thus, it can be
inferred that previously published reports of underpredictions of the
magnitude of DDIs perpetrated by paroxetine are explained by appli-
cation of a competitive inhibition model for a mechanism-based
enzyme inactivator. Nonlinear accumulation of paroxetine was also
predicted by the model with an accuracy greater than 90%, suggesting
that autoinactivation of CYP2D6 rather than reversible saturation of
the enzyme may largely explain this pharmacokinetic phenomenon.

Nonspecific microsomal binding has been shown to significantly
impact the determination of metabolic intrinsic clearance and enzyme
kinetic parameters, with its consideration in the prediction of human
clearance from in vitro metabolism data reproducibly improving pre-
dictive accuracy (Obach, 1997, 1999, 2000; Venkatakrishnan et al.,
2000). More recently, it has been demonstrated that nonspecific
microsomal binding can account for underestimation of inhibitor
potency (i.e., overestimation of IC_{50} or K_{i} values) when dealing with
lipophilic basic perpetrator drugs (Tran et al., 2002; Margolis and
Obach, 2003), with the potential implication being erroneous under-
estimation of drug interaction risk. The risks of not considering
nonspecific microsomal binding in estimating inhibitory potency may
be greater when dealing with mechanism-based inactivators, due to
the relatively high microsomal concentrations that are typically used
during preincubation of the inactivator in these experiments. In this
investigation, application of a "total" K_{i} based on nominal inhibitor
concentrations added in vitro resulted in a gross underestimation of
the magnitude of drug-drug interactions with several CYP2D6 sub-
strates, in contrast to a 90% accurate prediction on average when an
"unbound" K_{i} estimate was used. When nonspecific binding was not
considered, despite application of a MBI model that accounts for
time-dependent loss of enzyme activity, a <2-fold increase in victim
drug AUC is predicted upon paroxetine coadministration, signifi-
cantly underestimating the observed 5- to 8-fold magnitudes of clin-
ical interactions of paroxetine with CYP2D6 substrates.

A key assumption of the scaling model used here is that enzyme
inactivation is the sole mechanism of these DDIs, since the reversible
component of CYP2D6 inhibition by paroxetine is not considered.
Furthermore, it is also assumed that the effect of paroxetine is sub-
strate-independent. This is based on the assumption that the MBI
completely prevents substrate binding in a productive orientation at
the enzyme active site(s), such that metabolic capacity toward all
substrates would be equally affected. Atypical kinetics of dextro-
methorphan metabolism by CYP2D6 have been described (Yu et al.,
2001), suggesting that multiple binding orientations are possible.
Thus, the theoretical possibility remains that the assumption of sub-
strate independence of K_{i} and k_{inact} of mechanism-based inactivation
of CYP2D6 by paroxetine may not be true, if the MBI results in an
orientation-specific (and hence substrate-dependent) loss of substrate
binding at the enzyme active site. Nevertheless, paroxetine reproduc-
ibly impairs the clearance of structurally diverse CYP2D6 substrates,
which would suggest that the likelihood of paroxetine producing
substrate-specific CYP2D6 inhibition is small and of limited clinical
relevance.

The lack of information on the in vivo degradation rates of many

![Fig. 4. Analysis of the sensitivity of the MBI scaling model-predicted drug-drug interaction magnitude (fold-increase in victim drug AUC) to the estimated CYP2D6
degradation half-life for victim drugs with f_{2D6} values ranging from 0.5 to 1.0, using eqs. 2 to 4. Note that the use of a 14-h estimate of CYP2D6 half-life (as opposed to
the 51-h estimate) results in an approximately 2-fold underestimation of interaction magnitude for victim drugs examined in this investigation (mean f_{2D6} = 0.87).](image)
human drug-metabolizing P450s represents a significant limitation that increases the uncertainty in risk assessment, even when the desired in vitro data are available. In the present investigation, we have utilized an indirect approach of pharmacokinetic deconvolution to estimate the half-life of CYP2D6 in humans as ~51 h. A comparable mean half-life estimate of 50 h (range 25–80 h) has been reported for human CYP2E1 by measuring the time course of return of chloroazoxone 6-hydroxylation clearance to baseline following administration of the rapidly cleared CYP2E1 inactivator disulfiram (Emery et al., 1999). Thus, the estimated CYP2D6 half-life of 51 h is in excellent agreement with the degradation half-life estimated for another human P450 isoform within the same family, using a similar indirect approach. When information on enzyme half-life is not available, compound-specific analysis of the sensitivity of the IVIVE model to $k_{\text{degr}}$ represents a useful approach to estimating the level of confidence in the predicted DDI magnitude. The same is applicable when there is uncertainty in the estimated enzyme-available inhibitor levels due to possible active hepatic uptake (von Moltke et al., 1998) or transient high concentrations during first pass through the liver (Ito et al., 1998; Kanamitsu et al., 2000).

In the current retrospective analysis, the possibility of concentrative hepatic uptake of paroxetine has not been considered due to the lack of data on the extent of hepatocellular uptake of paroxetine, or identified biochemical mechanisms of active hepatic uptake transport of this drug. Paroxetine displays extensive hepatic distribution, with a liver/plasma total concentration ratio of ~26 (von Moltke et al., 1995). However, it is unknown whether this extent of distribution is significantly in excess of that expected from the relative extents of plasma and hepatic tissue binding. The choice of inhibitor concentration (systemic unbound versus systemic total versus estimated maximum unbound concentration at the inlet to the liver) did not significantly influence the predicted drug interaction magnitudes in the current retrospective analysis because the limits of the range of these alternative exposure estimates following clinical doses of paroxetine were outside the dynamic range of the sensitivity curve for these victim drugs. Thus, a true test of the applicability of $I_{\text{a,unb}}$ of paroxetine as a surrogate of enzyme-available concentrations will require DDI studies using subtherapeutic doses that yield exposures in the dynamic range of the sensitivity curve. The successful prediction of paroxetine DDI in the current analysis therefore does not necessarily support the general applicability of unbound systemic concentrations of an inhibitor in the prediction of interaction magnitude. Although the application of unbound systemic exposures is theoretically appealing and has been successful for many structurally distinct mechanism-based inactivators (Mayhew et al., 2000; Wang et al., 2004; Ernest et al., 2005), the successful prediction of paroxetine (30 mg q.d.) would produce a 14-fold increase in victim drug AUC. Thus, when MBI is suspect, risk assessment is not straightforward. It requires thorough kinetic analysis and IVIVE using the appropriate scaling model (Mayhew et al., 2000), with consideration of factors such as microsomal binding in vitro, to enable unbiased estimation of inhibitor potency, to ultimately increase confidence in clinical DDI risk assessment from in vitro data.

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References