A Novel Model for the Prediction of Drug-Drug Interactions in Humans Based on in Vitro Cytochrome P450 Phenotypic Data

Chuang Lu, Gerald T. Miwa, Shimoga R. Prakash, Liang-Shang Gan,1 and Suresh K. Balani

Drug Metabolism and Pharmacokinetics, Drug Safety and Disposition, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts

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ABSTRACT:

Ketoconazole has generally been used as a standard inhibitor for studying clinical pharmacokinetic drug-drug interactions (DDIs) of drugs that are primarily metabolized by CYP3A4/5. However, ketoconazole at therapeutic, high concentrations also inhibits cytochromes P450 (P450) other than CYP3A4/5, which has made the predictions of DDIs less accurate. Determining the in vivo inhibitor concentration at the enzymatic site is critical for predicting the clinical DDI, but it remains a technical challenge. Various approaches have been used in the literature to estimate the human hepatic free concentrations of this inhibitor, and application of those to predict DDIs has shown some success. In the present study, a novel approach using cryopreserved human hepatocytes suspended in human plasma was applied to mimic the in vivo concentration of ketoconazole at the enzymatic site. The involvement of various P450s in the metabolism of compounds of interest was quantitatively determined (reactive phenotyping). Likewise, the effect of ketoconazole on various P450s was quantitated. Using this information, P450-mediated change in the area under the curve has been predicted without the need of estimating the inhibitor concentrations at the enzyme active site or the Km. This approach successfully estimated the magnitude of the clinical DDI of an investigational compound, MLX, which is cleared by multiple P450-mediated metabolism. It also successfully predicted the pharmacokinetic DDIs for several marketed drugs (theophylline, tolbutamide, omeprazole, desipramine, midazolam, alprazolam, cyclosporine, and loratadine) with a correlation coefficient (r2) of 0.992. Thus, this approach provides a simple method to more precisely predict the DDIs for P450 substrates when coadministered with ketoconazole or any other competitive P450 inhibitors in humans.

Ketoconazole is an antifungal medicine and is also a potent CYP3A4/5 (CYP3A4) inhibitor in humans. Ketoconazole is widely used in vitro as a selective CYP3A4 inhibitor at low concentrations (Pelkonen et al., 1998; Li et al., 1999). However, at high concentrations it also inhibits cytochrome P450 (P450) isoymes other than CYP3A4 (Venkatakrishnan et al., 2001; Stresser et al., 2004) and UDP glucuronosyltransferase (Yong et al., 2005), as well as transporters such as sodium taurocholate cotransporting polypeptide, P-glycoprotein (Pgp), breast cancer resistance protein, and multidrug resistance-associated protein 2 (Azer et al., 1995; Salphati and Benet 1998, Achira et al., 1999; Xia et al., 2005b). For the purpose of overcoming possible drug resistance, clinical usage of ketoconazole involves much higher concentrations (~25-fold) than its in vitro efficacious concentration (Schaefer-Korting et al., 1984). For studying the worst-case scenario of CYP3A4-mediated drug-drug interaction (DDI) potential on a compound, dosing of ketoconazole at 200 mg b.i.d. is recommended by the Pharmaceutical Research and Manufacturers of America (Bjornsson et al., 2003). At such a high dose, the maximum plasma concentration (Cmax) could reach as high as 20 μM (Rochlitz et al., 1988; Hsu and Chen, 1997). Other dose regimens, such as 200 mg/day, have also been reported. These doses showed plasma Cmax ranging from 3.2 to 10 μM with most of the values about 10 μM (Schaefer-Korting et al., 1984; Ito et al., 1998; Pelkonen et al., 1998; Hardman et al., 2001; Blanchard et al., 2004). Ketoconazole treatment has been found to completely inhibit CYP3A4 activity in vivo (Obach et al., 2006), but the cross-inhibition to P450s other than CYP3A4 has not yet been thoroughly shown in vivo as it has been in vitro. The cross-P450 isozyme inhibition by ketoconazole in vivo is usually overlooked when predicting DDIs between ketoconazole and CYP3A4 substrates, and ketoconazole is still treated as a selective CYP3A4 inhibitor in vivo.

P450-reactive phenotyping is a quantitative measurement of the relative contributions of each P450 to the overall metabolism of a drug, when this drug is primarily metabolized by P450s. Several methods are commonly used in the determination of the reactive phenotyping that include the relative activity factor (Crespi, 1995; Venkatakrishnan et al., 2001), chemical inhibitors (Newton et al., 1995; Bourrie et al., 1996; Lu et al., 2003), and monoclonal antibodies (mAbs) (Gelboin et al., 1999; Shou et al., 2000; Soars et al., 2003). In

ABBREVIATIONS: CYP3A4, CYP3A4/5; P450, cytochrome P450; Pgp, P-glycoprotein; DDI, drug-drug interaction; mAb, monoclonal antibody; AUC, area under the curve; KHB, Krebs-Henseleit buffer; LC/MS/MS, liquid chromatography/tandem mass spectrometry; HPLC, high-performance liquid chromatography; fao, fraction of activity remaining of a given enzyme in the presence of inhibitor; fm,P450, fraction of metabolism by a given enzyme; MLX, Millennium investigational compound X.
one recent report, all three methods were applied to determine the relative P450 contributions to the metabolism of a proteasome inhibitor, Velcade (Millennium Pharmaceuticals, Cambridge, MA) (Uttamsingh et al., 2005). With the introduction of the potent and selective CYP2C19 and CYP3A4 inhibitors benzylviranol and azamulin, respectively (Walsky and Obach 2003; Streser et al., 2004), using chemical inhibitors to determine the reactive phenotyping has become an easy and cost-effective choice.

A major and important effort in the pharmaceutical industry is predicting the clinical DDI from in vitro data. It is especially important for drugs that are primarily metabolized by CYP3A4 because many drugs on the market are CYP3A4 inhibitors/substrates. Besides the pharmacokinetic properties of the substrate drug, two factors that dictate the DDI potential are the inhibitor’s inhibition constant ($K_i$) and the enzyme site free concentration of that inhibitor. The $K_i$ usually can be determined from in vitro microsomal incubation assays with the consideration of protein binding. But the enzyme site inhibitor concentration cannot readily be assessed. Vast efforts have been focused on defining the enzyme site concentration, such as use of the peripheral vein concentration, the portal vein concentration, the steady-state systemic plasma concentration, a multiple (such as 10×) of the plasma concentration, the maximum plasma concentration, the maximum hepatic input concentration, or the liver concentration (Blanchard et al., 2004; Cook et al., 2004; Ito et al., 2004; Bachmann 2006; Obach et al., 2006). For each possible concentration, there is always a choice of using the total concentration or the free concentration. Choosing one way to estimate the enzyme site inhibitor concentration may provide a better DDI prediction for certain classes of drugs. However, there is no consensus on which concentration represents the enzyme site concentration and therefore should be used for the DDI prediction of all the classes of drugs.

In the present study, human hepatocytes were incubated in human plasma in the presence of various concentrations of ketoconazole to determine the ketoconazole cross-reactivity or cross-inhibition on five major P450s: CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Reactive phenotyping was determined for the investigational compound MLX using chemical inhibitors and mAbs. Without the need to consider the enzyme site inhibitor concentration or the $K_i$, the DDI potential [the area under the curve (AUC) changes] was predicted using the combined information from the ketoconazole cross-reactivity in human hepatocytes and the reactive phenotyping of MLX. This method was also applied to a set of marketed drugs and showed a good correlation between the predicted DDI and the observed clinical DDI. Thus, this method can help predict DDIs early in the preclinical development stage (Balani et al., 2005).

**Materials and Methods**

**Reagents.** Pooled human liver microsomes from 50 donors were purchased from XenoTech LLC (Kansas City, KS). Cryopreserved human hepatocytes were purchased from In Vitro Technologies, Inc. (Baltimore, MD) and AP Sciences Inc. (Baltimore, MD). 4-Hydroxybutabutamide, 4-hydroxyphenytoin, dextrophan, 1'-hydroxymidazolam, benzylviranol, and azamulin were purchased from BD Gentest (Woburn, MA). Phenacetin, acetaminophen, tolbutamide, dextromethorphan, and azamulin were purchased from In Vitro Technologies, Inc. (Baltimore, MD) and AP Sciences Inc. (Hicksville, NY).

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selective inhibitors and probe substrates were 100 μM furafylline and 30 μM phencacetin for CYP1A2, 5 μM sulfaphenazole and 150 μM tolbutamide for CYP2C9, 20 μM benzylnirvanol and 100 μM S-mephenytoin for CYP2C19, 5 μM quinidine and 8 μM dextromethorphan for CYP2D6, and 2 μM azamulin and 50 μM testosterone for CYP3A4. The analyses of the P450 substrate metabolites were the same as described earlier. The analysis of MLX metabolites were performed using an HPLC system consisting of an Agilent 1100 binary pump (Palo Alto, CA) coupled to an IN/US β-RAM detector (IN/US Systems, Inc., Tampa, FL). A 60-min gradient with 1.0 ml/min flow rate was applied to separate the metabolites from their parent peak using a Synergi Fusion-RP column (4.6 × 250 mm, 4 μm, 80 Å) purchased from Phenomenex Inc. (Torrance, CA). The peak areas of MLX and its metabolites were detected using an on-line β-RAM detector with 1:1 (v/v) infusion of the In-Flow Scintillator (IN/US Systems, Inc.). The sample injection volume was 100 μl. Mobile phases A and B were water and acetonitrile, respectively, each supplemented with 0.1% v/v formic acid.

**Reactive Phenotyping of MLX Using Anti-P450 mAbs.** Similar to the chemical inhibitor assay, predetermined dilutions of mAbs were preincubated with microsomes (0.5 mg/ml) at 37°C for 10 min. The [14C]MLX (10 μM) and NADPH (2.0 mM)/MgCl2 (3 mM) were then added to start the incubations at 37°C for 15 min. The hen egg white lysozyme (control protein in the same -fold of dilutions) was included in the study as control for the 100% P450 metabolism (i.e., fAmax = 1), a prediction value of 2.74-fold increase in AUC was calculated. A similar calculation was done for another non-omeprazole study in which a low ketoconazole dose of 50 mg q.d. was reported (University of Washington DDI database, 2004). This predicted for a 1.74-fold change in AUC (Table 5). For theophylline and alprazolam, the renal clearance had marginal contribution to the total clearance of theophylline and alprazolam, but the information on ketoconazole’s effect on renal clearance was not available. Therefore, a range of values was predicted such that the low value considers the renal clearance contribution in the calculation, whereas the high value does not. One can imagine the low value is similar to the situation in which renal clearance is not affected by ketoconazole, whereas the high value is similar to the situation in which renal clearance is totally blocked by ketoconazole.

**Results**

The inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by ketoconazole in human hepatocytes in KHB or human plasma is presented in Tables 1 and 2. Figure 1 shows the plot of extracellular ketoconazole concentration versus the P450 activity remaining from the data in Table 2. For both Tables, column 1 represents the concentrations in the incubation—the initial concentration of ketoconazole. The “Keto Extracellular” (column 2) represents the extracellular concentration of ketoconazole remaining in the KHB buffer or plasma after the equilibration period.

**TABLE 2**

<table>
<thead>
<tr>
<th>Koncentracion in</th>
<th>Koncentracion Extracellular</th>
<th>fA (%)*</th>
<th>CYP1A2</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 μM</td>
<td>71.8</td>
<td>79</td>
<td>60</td>
<td>32</td>
<td>96</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>50 μM</td>
<td>34.0</td>
<td>81</td>
<td>71</td>
<td>42</td>
<td>84</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25 μM</td>
<td>14.1</td>
<td>83</td>
<td>72</td>
<td>57</td>
<td>59</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12.5 μM</td>
<td>7.30</td>
<td>89</td>
<td>75</td>
<td>63</td>
<td>91</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6.25 μM</td>
<td>4.04</td>
<td>92</td>
<td>80</td>
<td>83</td>
<td>99</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3.13 μM</td>
<td>2.22</td>
<td>92</td>
<td>84</td>
<td>90</td>
<td>100</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1.6 μM</td>
<td>0.857</td>
<td>97</td>
<td>79</td>
<td>89</td>
<td>97</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*a fA (%) = fraction of enzyme activity remaining in the presence of ketoconazole inhibition.*

Ketoconazole was equilibrated with human hepatocytes in human plasma to allow nonspecific binding. After equilibration, the extracellular concentration of ketoconazole was measured. The remaining P450 activities in hepatocytes (fA) were also determined using the probe substrates.
where A, B, C, D, and E are the percentage contributions of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, respectively, to the metabolism of MLX.

Comparing column 1 with column 2, there was less than 20% of ketoconazole remaining in the KHB incubation, whereas in plasma incubation there was about 70% of ketoconazole remaining. The lower extracellular concentrations attribute to nonspecific binding to the hepatocytes. Ketoconazole, on incubation with KHB in the traditional way, showed a dose-dependent inhibition of all five P450s with the rank order of CYP3A4 > CYP2C9 > CYP2C19 > CYP1A2 > CYP2D6. When human hepatocytes were incubated in human plasma instead, a system aiming to mimic the in vivo condition, ketoconazole showed lower inhibition, whereas the rank order remained similar: CYP3A4 > CYP2C19 > CYP2C9 > CYP1A2 > CYP2D6. In contrast to the incubation in KHB, it was noticed that at high concentrations, ketoconazole tended to cause more inhibition of CYP2D6 than CYP2C9. Within the concentration range tested in this study, dose-dependent inhibition of ketoconazole on CYP2D6 was not observed in the presence of plasma.

As expected, ketoconazole was found to be stable in the hepatocyte incubations in KHB or plasma during the 30-min period of equilibration (data not shown); therefore, the lower extracellular concentrations of ketoconazole concentrations were caused by binding instead of metabolism. Table 3 shows the reactive phenotyping results for MLX using chemical inhibitors, suggesting MLX is primarily metabolized by CYP3A4, followed by CYP2D6 and to a lesser extent by CYP1A2. These results were similar to those generated using mAbs, except the contribution of CYP2D6 in the chemical inhibitor assay was much higher. In our experience, using quinidine and dextromethorphan tended to overestimate CYP2D6 contribution compared with that using the relative activity factor or the mAb method (Uttamsingh et al., 2005). A hypothesis that may help explain this observation is that quinidine at low concentrations is known to stimulate CYP3A4 metabolism, but at high concentrations it appears to show inhibition (Ludwig et al., 1999; Ngui et al., 2000). This stimulation by quinidine could reduce the estimates for net CYP3A4 inhibition, thereby leading to underestimation of the cross-reactivity of quinidine on CYP3A4, which in turn would lead to an overestimation of CYP2D6 contribution.

Table 4 presents the cross-reactivity of the P450 inhibitors used in this study and a method of correcting for this cross-reactivity for obtaining the reactive phenotyping results of MLX. For example, the observed inhibition of MLX metabolism by furafylline (Table 4, the rightmost column) is attributed to the sum of partial inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by furafylline, measured using P450 probe substrates. An equation can then be derived for each inhibitor. By solving the five equations simultaneously, the contribution of the five P450s (the five variables) to the metabolism of MLX can be solved, and the results are shown in Table 3. Table 5 lists DDI predictions for the investigational compound (MLX) and a set of marketed compounds. Also included is a comparison between the values predicted from this study and the values observed in clinical trials. A correlation coefficient (r²) of 0.992 (Fig. 2) was derived from the data in Table 5. A slope close to unity (0.980) suggested a good prediction of clinical DDIs from the in vitro data.

**Discussion**

Cryopreserved hepatocyte suspension is a valid model for studying drug metabolism and DDIs. Hepatocyte incubations are usually carried out in KHB, which is an essential but minimum salt buffer.

**TABLE 3**

<table>
<thead>
<tr>
<th>Chemical inhibitors</th>
<th>CYP1A2</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td>4.8</td>
<td>0.0</td>
<td>0.0</td>
<td>21.0</td>
<td>87.0</td>
</tr>
</tbody>
</table>

\[ f_{\text{in,exo}}(\%) = \text{fractional contribution of specific P450 to the metabolism of MLX.} \]

**TABLE 4**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>CYP1A2 Phenacetin</th>
<th>CYP2C9 Tolbutamide</th>
<th>CYP2C19 S-Mephenytoin</th>
<th>CYP2D6 Dextromethorphan</th>
<th>CYP3A4 Testosterone</th>
<th>average % inhibitionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Furfuryline</td>
<td>85.5</td>
<td>6.2</td>
<td>53.1</td>
<td>7.5</td>
<td>5.3</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Sulfaphenazole</td>
<td>5.4</td>
<td>92.8</td>
<td>13.8</td>
<td>19.5</td>
<td>15.0</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Benzylnirvanol</td>
<td>0.0</td>
<td>43.6</td>
<td>97.0</td>
<td>10.5</td>
<td>33.3</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Quinidine</td>
<td>-44.5</td>
<td>22.4</td>
<td>28.3</td>
<td>66.6</td>
<td>23.2</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Azamulin</td>
<td>-75.3</td>
<td>15.3</td>
<td>17.6</td>
<td>7.5</td>
<td>89.9</td>
</tr>
</tbody>
</table>

\[ ^a \text{The relative contribution of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 to the metabolism of MLX was calculated by the following equations:} \]

\[ \begin{align*}
8.54 &= 0.855a + 0.062c + 0.531c + 0.075d + 0.053e \\
3.57 &= 0.054a + 0.928b + 0.138c + 0.195d + 0.154e \\
23.4 &= 0.0a + 0.315b + 0.530c + 0.105d + 0.333e \\
30.4 &= 0.0a + 0.224b + 0.283c + 0.666d + 0.232e \\
77.6 &= 0.0a + 0.153b + 0.176c + 0.075d + 0.899e
\end{align*} \]

\[ \text{where A, B, C, D, and E are the percentage contributions of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, respectively, to the metabolism of MLX.} \]

\[ ^b \text{A negative number may suggest a stimulation of the metabolism. Negative values of quinidine and azamulin inhibition on CYP1A2 were also used in the calculation, and the results were similar to those in Table 3.} \]
for hepatocytes to maintain their metabolic function. Several researchers (Shibata et al., 2002; Bachmann et al., 2003) have tried to add human serum into hepatocyte incubations to better mimic the in vivo conditions. Cryopreserved hepatocytes are known to preserve the metabolic function. Several researchers (Shibata et al., 2002; Bachmann et al., 2003) have tried to add human serum into hepatocyte incubations to better mimic the in vivo conditions. Cryopreserved hepatocytes are known to preserve the metabolic function.

TABLE 5

Marketed compounds: ketoconazole DDI prediction using the hepatocytes in plasma model

<table>
<thead>
<tr>
<th>Compound</th>
<th>Predicted*</th>
<th>Observed</th>
<th>f&lt;sub&gt;real&lt;/sub&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLX</td>
<td>8.33</td>
<td>*</td>
<td>0.18</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Theophylline</td>
<td>1.13–1.16</td>
<td>1.11</td>
<td>0.18</td>
<td>Obach 2005*</td>
</tr>
<tr>
<td>Desipramine</td>
<td>1.00</td>
<td>1.02</td>
<td>0.70</td>
<td>Obach 2005*</td>
</tr>
<tr>
<td>Midazolam</td>
<td>16.7</td>
<td>17.0</td>
<td>&lt;0.01</td>
<td>Obach 2005*</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>1.43</td>
<td>1.77</td>
<td>0.001</td>
<td>Obach 2005*</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1.74–2.74</td>
<td>1.36–2.05</td>
<td>&lt;0.01</td>
<td>Soars 2003*</td>
</tr>
<tr>
<td>Loratadine</td>
<td>2.48</td>
<td>3.47</td>
<td>Negligible</td>
<td>U of Washington DDI database, Soars 2003*</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>3.45</td>
<td>4.39</td>
<td>&lt;0.01</td>
<td>U of Washington DDI database, Soars 2003*</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>2.75–4.91</td>
<td>3.98</td>
<td>0.20</td>
<td>U of Washington DDI database, Soars 2003*</td>
</tr>
</tbody>
</table>

* Predictions are based on our model assuming 200 mg b.i.d. doses of ketoconazole have plasma C<sub>max</sub> of 10 μM and linear PK.

The DDI potential is usually expressed as the ratio of AUC<sub>I</sub> (AUC when the inhibitor is present) to AUC<sub>ref</sub> (AUC when the inhibitor is absent). The ratio can be simplified as a fraction of hepatic metabolism (f<sub>hep</sub>) if the substrate is administered i.v. or metabolism by intestinal or other organs is insignificant compared with that of the liver. Similar to what was proposed by Ito et al. (2005), in the presence of an inhibitor, eq. 1 can be rewritten as:

\[
CL_{i} = f_{\text{hep}} \left( \frac{f_{\text{m,3A4}} CL}{K_{3A4}} + \frac{f_{\text{m,2C9}} CL}{K_{2C9}} + \ldots \right) + f_{\text{other}} CL
\]
AUC1
AUC = CL
= f_{m,hep} \left[ \frac{f_{m3A4}}{1 + \frac{1}{K_{3A4}}} + \frac{f_{m2C9}}{1 + \frac{1}{K_{2C9}}} + \ldots \right] + f_{other}/f_{other} (3)

Thus, the AUC ratio for a compound primarily cleared by metabolism can be expressed as a function of the remaining activity of each enzyme toward the metabolism of this compound, or the remaining clearance routes.

AUC1
AUC = CL
= f_{m,hep}(f_{m3A4}f_{3A4} + f_{m2C9}f_{2C9} + \ldots) + f_{other}/f_{other} (4)

where $f_{m,P450}$ is again the relative contribution of an enzyme to the total metabolism of a compound (without inhibitor), and $f_A$ is the fraction of enzyme activity remaining in the presence of an inhibitor [i.e., $f_{A,hep} = 1/(1 + 1/K_{3A4})$]. Whereas $f_{m,P450}$ could be determined in several ways (Uttamsingh et al., 2005), including using the selective chemical inhibitors (Table 3), the $f_A$ in vivo can be estimated from the $f_A$ obtained from the in vitro data such as those in Table 2. Thus, DDI can be predicted by combining the information from the reactive phenotyping study and the inhibitor’s cross-reactivity study (eq. 4 if a compound is primarily cleared by metabolism or the other clearance route is known).

The $C_{max}$ of ketoconazole at the steady state can be used to assess the worst-case inhibitory effect, although the $C_{max}$ may vary from person to person because of interindividual variability in $pK$ or from study to study because of different dosing regimens. For an individual $C_{max}$ or a comparable in vitro concentration $x$ in our model, the fraction of enzyme activity remaining ($f_A$) can be calculated from the titration curves (Fig. 1 or Table 2). Theoretically, the $f_A$ and $x$ follow a sigmoidal relationship:

$$f_A = \frac{(\text{Maximum activity} - \text{Minimum activity})}{1 + 10^{(x - \text{log EC50})*\text{Hill slope}}} \ldots (5)$$

Practically, to determine the percentage of enzyme activity remaining $f_A$ at an inhibitor concentration $x$ requires a well fitted sigmoidal curve. From our data, within the physiological concentration range, ketoconazole only partially inhibits common P450s in human hepatocytes in the presence of human plasma. Thus, a linear model is more practical for estimating the percentage remaining of enzyme activity $f_A$ at an inhibitor concentration $x$. This linear model assumes that within a narrow range (one concentration above and one concentration below the targeted $x$, $C_{max}$), the inhibition response is linear. Therefore, the P450 activity remaining can be interpreted between these points:

$$f_A = A_1 - (x - C_1)(A_2 - A_3)/(C_2 - C_1) \ldots (6)$$

where $A_1$, $A_2$, and $C_1$, $C_2$ are the activities and concentrations above and below the target concentration $x$, respectively.

MLX is an investigational compound. Through qualitative enzyme mapping and metabolite profiling studies, MLX was found to be metabolized mainly by P450 isozymes. Furthermore, quantitative P450 phenotyping also found that this compound was mainly metabolized by CYP3A4 with some contributions from CYP2D6 and CYP1A2. Considering that CYP2D6 contribution may be overestimated, ketoconazole at a concentration of 10 to 20 μM inhibits 87% of the CYP3A4 contribution (∼100% inhibition of the 87% CYP3A4 contribution) (Tables 2 and 3) and 1% of the CYP1A2 contribution (∼20% cross-reactivity of the 4.8% CYP1A2 contribution) (Tables 2 and 3). Thus, using the approach of DDI prediction illustrated in eq. 4, an 8.33-fold increase in AUC was predicted for MLX coadministration with ketoconazole. A clinical DDI study using 200 mg b.i.d. of ketoconazole showed an increase of mean AUC of MLX within 10% of the predicted 8.33-fold (unpublished data).

There is limited literature available on compounds with information on both clinical DDIs with ketoconazole and quantitative reactive phenotyping. Table 5 lists several compounds found in recent literature (University of Washington DDI database handout, 2004; Galetin et al., 2006; Obach et al., 2006) with the observed clinical DDI. Although there is no reactive phenotyping information available for theophylline and desipramine, these two compounds have been treated as prototypical substrates for CYP1A2 and CYP2D6 for retroactive prediction based on our method. Midazolam is widely considered as an exclusive CYP3A4 substrate. Omeprazole was found to be metabolized by CYP2C19 (60%) and CYP3A4 (40%), whereas tolbutamide was found to be metabolized by CYP2C9 (72%) and CYP2C19 (28%) (Soars et al., 2003). CYP3A4 was reported to contribute 71, 80, and 60% to the metabolism of cyclosporine, alprazolam, and loratadine, respectively (Galetin et al., 2006), but the rest of the clearance routes have not been documented. It is also known that cyclosporine is partially cleared renally and that CYP2D6 contributes toward the metabolism of loratadine (Yumibe et al., 1996). In this study, the prediction of these three compounds was based on the assumption that ketoconazole does not affect the non-CYP3A4 metabolic clearance routes. In vivo human plasma ketoconazole concentration values vary in the literature, most being around 10 μM with 20 μM being the highest at 200 mg b.i.d.; in practice, it is assumed to be 10 μM. The observed clinical values were adapted from Obach et al. (2006) and the DDI database (handout) from the University of Washington (2004). Using this information and the ketoconazole cross-reactivity data in Table 2, the prediction of AUC changes is presented in Table 5. It is noteworthy that these predictions compare very closely with the observed clinical values ($r^2 = 0.992$ and slope of 0.980) (Fig. 2). For most of the compounds, renal clearance plays a minor role (1% or less) (Table 5); thus, renal clearance contribution was not included in the calculation. For theophylline and alprazolam, predictions were made with and without considering the renal clearance contribution. Because the effect of ketoconazole on the renal clearance of these two compounds is unknown, a range of predictions was reported such that the low value shows ketoconazole has no effect on renal clearance, whereas the high value shows that ketoconazole totally blocked the renal clearance. For Fig. 2, average values were used for data presented as ranges in Table 5. Desipramine was treated as a selective CYP2D6 substrate. Ketoconazole was predicted to have no effect on its metabolic clearance based on our hepatocyte cross-reactivity study. Prediction of ketoconazole effect on its renal clearance was not explored. For loratadine, cyclosporine, and alprazolam, only $f_{m,P450}$ information was available (Galetin et al., 2006); hence, the interaction could be underestimated.

In summary, the DDI prediction approach presented in this study involves using two in vitro determinations: the reactive phenotyping (the relative contributions of enzymes to the overall metabolism of a drug, an intrinsic property of the drug) and the P450 cross-reactivity of an inhibitor at close to the physiological condition (hepatocyte
incubation in human plasma). Ketoconazole is used in this study as an example. However, most inhibitors of interest, except those that are efflux pump substrates that can be pumped out of hepatocytes leading to reduced hepatocyte concentration and hence reduced potency, can be applied. In most cases, the pharmaceutical industry likes to show the worst-case scenario for DDI potential for drugs that are mainly metabolized by a few isozymes; therefore, a handful of known inhibitors, such as ketoconazole for CYP3A4 and quinidine for CYP2D6, are often used. Thus, this approach provides a simple way of predicting the DDI early in the preclinical development stage, with the main advantage being not having to estimate the actual inhibitor concentration at the enzyme site.

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References


