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A Novel Approach to the Prediction of Drug-Drug Interactions in Humans Based on the Serum Incubation Method

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Summary: A novel method for the prediction of drug-drug interaction has been established based on the in vitro metabolic stability in the “serum incubation method” using cryopreserved human hepatocytes suspended in 100% human serum. As a novel approach, the inhibitory effect of inhibitors on the metabolism of substrates during the first-pass elimination process in the liver (hepatic availability) and in the elimination process from the systemic circulation (hepatic clearance) were separately predicted with the anticipated inhibitor/substrate concentrations during absorption and in the systemic circulation, respectively. Ketoconazole strongly inhibited CYP3A4-mediated terfenadine metabolism in vitro, and the method predicted 6- to 37-fold increase of terfenadine AUC by the concomitant dosing of ketoconazole, which reasonably well agreed with the observed 13- to 59-fold increase of AUC in clinical studies. The CYP3A4-mediated metabolism of indinavir was also subject to the inhibition by ketoconazole in vitro at the lower indinavir concentration (2 μM), whereas no substantial inhibition was observed at 12 μM due to the saturation of indinavir metabolism. Predicted no interaction between ketoconazole and indinavir was consistent with the minimal increase (1.3-fold increase) of indinavir AUC by ketoconazole observed in clinical study. In addition, the method was applied to the CYP2D6-mediated desipramine-quinidine interaction: the predicted 6.4-fold increase of desipramine AUC by quinidine was consistent with the observed 6.7-fold increase of AUC in the clinical drug-drug interaction study. On the other hand, desipramine metabolism was little affected by ketoconazole in vitro, and consequently, it predicted no drug-drug interaction between desipramine and ketoconazole in humans, which agreed with the negligible interaction observed in clinical study. The accuracy of predictions for drug-drug interaction by the serum incubation method was evaluated by comparing the predicted increase of AUC after an oral administration of the inhibitor with the corresponding drug-drug interaction reported from clinical studies. These data demonstrated that the newly established method provides an in vitro tool for the prediction of drug-drug interaction with the accuracy ranging from 0.46 to 1.5.

Keywords: human hepatocytes; CYP3A4; CYP2D6; drug-drug interaction; terfenadine; ketoconazole; indinavir; desipramine; quinidine; availability; clearance; serum incubation method

Introduction

Drug-drug interaction has become one of the major concerns not only for medical providers during the treatment of patients but also for pharmaceutical industries during the development of new drugs.1) Serious adverse effects caused by drug-drug interaction have led to the withdrawal of many drugs, and regulatory agencies have expected decent information on both in vitro and in vivo drug-drug interactions in the compound registration dossier from the point of view to protect patients from adverse effects caused by drug-drug interactions.2) Either the termination of drug development based on the results from clinical drug-drug interaction studies, the severe prescribing restriction or the withdrawal of launched drug from the market causes an enormous economic loss for pharmaceutical companies. In addition, the clinical drug-drug interaction studies, routinely conducted at early phase in clinical development, may cause safety concerns for the volunteers if the drugs tested have narrow safety margins. Therefore, the inhibitory potential of drug candidates to drug-metabolizing enzymes including cytochrome P450s is routinely evaluated at early discovery stage for the timely optimization to circumvent
potential liability by structure modification.

It remains controversial whether the quantitative prediction of in vivo drug interaction from in vitro metabolic data is reliable or not.\(^1,3-4\) Uncertainties inherent in the conventional in vitro metabolism studies using human liver microsomes has been preventing quantitative prediction of in vivo drug interactions. For example, in vitro estimation of inhibitory potency in liver microsomes was in most cases affected by microsomal protein concentration, likely due to the specific and non-specific bindings of test compound to the microsomal proteins.\(^5-7\) Also, several controversial reports have been published whether the prediction can be more quantitative with unbound concentration in plasma\(^8-9\) or with total plasma concentration.\(^10-12\) Inaccurate estimations of inhibitory potency and/or plasma protein binding may partially explain the discrepancy between in vitro predictions and in vivo observations, whereas the possible involvement of hepatic uptake mediated by active transporters and/or the lack of important drug-metabolizing activities such as glucuronide/sulfate conjugations in liver microsomes cannot be ruled out as the cause(s) of discrepancy.\(^13-16\)

These uncertainties involved in the setting up of in vitro incubation system with human liver microsomes warranted further investigation of usefulness of cryopreserved human hepatocytes for drug-drug interaction as an alternative in vitro system.\(^17\) The “serum incubation method”, in which cryopreserved human hepatocytes are suspended in 100% human serum, has been established for the extrapolation of in vitro metabolic clearance to the in vivo values, and the prediction demonstrated reasonable agreements between predicted and observed hepatic clearance/availability in rat\(^18\) and human.\(^19\) Because the 100% serum containing test compound is used as an incubation medium in this method, separate experiments to evaluate unbound fraction of test compounds in the serum (plasma) are not necessary in the process of calculating hepatic clearance. It also has advantages over conventional in vitro systems using liver microsomes in the regard that the hepatocytes possess the capabilities of active transporter(s) and endogenous supplies for non-CYP drug metabolizing enzymes. The metabolic stability of test compound was determined in the presence of different concentrations of inhibitor in the cryopreserved human hepatocytes suspended in 100% serum. The obtained values were extrapolated to the hepatic clearance and availability by the procedure established in the serum incubation method.\(^19\) The extent of drug-drug interaction was predicted as the increase of AUC after oral administration of test compound by the concomitant dosing of inhibitor. The purpose of the present study was, therefore, to examine the predictability of serum incubation method for the pharmacokinetic drug-drug interactions in humans by comparing in vitro predictions with those reported from clinical drug-drug interaction studies.

**Materials and Methods**

**Materials:** Terfenadine, desipramine and quinidine were purchased from SIGMA (St. Louis, MO), and ketoconazole was from ICN Biomedicals (Aurora, OH). Diazepam and imipramine were obtained from Wako Pure Chemical Industries (Osaka, Japan). Indinavir was synthesized at Merck Research Laboratories (West Point, PA). Cryopreserved human hepatocytes (Lot 97 and 120) were purchased from In Vitro Technologies, Inc. (Baltimore, MD). Human serum was obtained from 3 healthy volunteers and stored at \(-80^\circ\text{C}\) until use.

**Incubation of cryopreserved human hepatocytes:** The value of scaling factor (SF) for the in vitro-to-in vivo extrapolation of metabolic clearance (as described in “Assumption and calculation” in the section of Materials and Methods) had been previously assessed\(^19\) for the pooled preparation (lot #97 and 120) of cryopreserved human hepatocytes used in the present study. The obtained scaling factor was \(1.23 \times 10^{10}\) living cells/kilogram of body weight (\(r^2 = 0.95\), data not shown). Both lots (#97 and #120) of human hepatocytes were prepared from extensive metabolizers for CYP2D6-mediated metabolism having high dextromethorphan O-demethylase activities according to the data provided by In Vitro Technologies, Inc (Baltimore, MD). The cell viability was measured by 0.4% trypan blue exclusion test, and the living cells were about 50% of total number of cells. The pH of human serum was adjusted to 7.4 at 37°C using 1-N HCl solution and placed on ice until use. Hepatocytes were re-suspended at either 1 \times 10^6 cells/mL (for the interaction study on terfenadine-ketoconazole), or 2 \times 10^6 cells/mL (for the interaction study on indinavir-ketoconazole, desipramine-quinidine and desipramine-ketoconazole) as a living cell in 100% human serum at ice-cold temperature. Suspensions of hepatocytes were pipetted into 1.5 mL eppendorf tubes at a volume of 760 \(\mu\text{L}\). Stock solution containing human serum at 100% was added to the suspension at a volume of 7.6 \(\mu\text{L}\) per tube. The final concentrations for the substrate and inhibitor in the interaction studies were designated as follows: terfenadine (substrate) at 0.02, 0.2 and 2 \(\mu\text{M}\) in the presence of 0, 0.04, 0.2, 0.6, 2 and 10 \(\mu\text{M}\) of ketoconazole (inhibitor); indinavir (substrate) at 2 and 12 \(\mu\text{M}\) in the presence of 0, 0.4, 2, 10 and 50 \(\mu\text{M}\) of ketoconazole (inhibitor); desipramine (substrate) at 0.1 and 0.5 \(\mu\text{M}\) in the presence of 0, 0.01, 0.1, 1 and 10 \(\mu\text{M}\) of quinidine (inhibitor); desipramine (substrate) at 0.1 and 0.5 \(\mu\text{M}\) desipramine in the presence of 0 and 10 \(\mu\text{M}\) of ketoconazole (inhibitor).

An aliquot (50 \(\mu\text{L}\)) of each sample was transferred to four 96-well plates with flat bottoms (n = 3), each of
which was used for the interaction studies or for the control samples. Incubations were carried out for the designated time periods at 37°C with shaking at 150 rpm under an atmosphere of 95% O2/5% CO2. After the incubation, samples were placed on ice, and the reaction was terminated by adding 150 μL of ice-cold ethanol solution containing 800 mM diazepam as an internal standard. The terminated samples were centrifuged (10,000 g × 10 min), and the compound in the supernatant was measured by LC-MS/MS method described in the following section.

**LC-MS/MS conditions:** The amount of compound remaining in the samples was determined by multiple reaction monitoring (terfenadine: 472.53 > 436.38, indinavir: 614.45 > 421.2, desipramine: 267.27 > 72.28, ketoconazole: 532.41 > 489.4, quinidine: 325.29 > 81.14, and diazepam: 285.18 > 193.36) in LC-MS/MS system Ultima VG (MICROMASS, Atlas park) connected with alliance 2700 HPLC system (Waters Corp). For HPLC, two mobile phases were prepared as lines A and B. The mobile phase A consisted of acetonitrile:water: 100 mM ammonium acetate (10%:80%:10%, v/v/v) and the mobile phase B consisted of acetonitrile:100 mM ammonium acetate (90%:10%, v/v). Two switching valves (NANOSPACE SI-2, Shiseido, Osaka) and another single line HPLC pump (PU-1580, JASCO, Tokyo) were also connected. A fast-gradient condition (3.5 min/cycle) was used at a high flow rate of 1 mL/min with a short column (CAPCELL PAK UG-120 2.0 × 10 mm Guard column, Shiseido, Osaka). Hundred percent of line A was initial condition. After an injection of sample (7 μL), the ratio of line A to line B was changed to 0% linearly for 0.5 min, and maintained it for next 1 min. The column was then washed for 0.9 min with acetonitrile:100 mM ammonium acetate (90%:10%, v/v) at a back flow rate of 1 mL/min by using another pump and switching valves. Thereafter, the condition was reset to the initial for preparing the next injection. The effluent was split with 0.2 mL/min, and the effluent from 0.5 to 1.5 min after the injection was introduced into the LC-MS/MS detector by using switching valves. The ratio of remaining substrate concentration to time zero (R) was calculated by dividing the peak-area ratio of compound to the internal standard in the incubated sample by the ratio in the control sample.

**Assumptions and calculations:** Average plasma (serum) concentrations of substrates and inhibitors derived from intestinal absorption (Cabs) was calculated as follows20–35 (Table 1), under the assumption that the absorption was completed at the time when the drug reached maximum plasma concentration in systemic circulation (Cs, max): Cabs = D/MW/(Tabs × 60 × QH × BW × RB) × 10^6 × F, where D, MW, Tabs, QH, BW, and RB represent oral dose (mg), molecular weight (g/M) [terfenadine 471.7; indinavir sulfate 613.8; desipramine hydrochloride 266.4; ketoconazole 531.4; quinidine sulf-
Table 2. Prediction of drug-drug interaction by serum incubation method and comparison with reported values

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Concentrations used in the serum incubation method (μM)</th>
<th>Predicted Values</th>
<th>Observed Increase of AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terfenadine</td>
<td>Ketoconazole</td>
<td>2</td>
<td>$F_{ht}$ 0.046</td>
<td>$F_{ht}/F_{ht}$ 5.0–13.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$F_{ht,1}$ 0.23–0.64</td>
<td>$CL_{ht}/CL_{ht,1}$ 1.3–2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>$CL_{ht}$ 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2–10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>$CL_{ht,1}$ 9–19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indinavir</td>
<td>Ketoconazole</td>
<td>12</td>
<td>$F_{ht}$ 0.71</td>
<td>$F_{ht}/F_{ht}$ 1.0–1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$F_{ht,1}$ 0.73–0.83</td>
<td>$CL_{ht}/CL_{ht,1}$ 1.3–1.7</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10–50</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>$CL_{ht}$ 16</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2–10&lt;sup&gt;f&lt;/sup&gt;</td>
<td>$CL_{ht,1}$ 11–14</td>
</tr>
<tr>
<td>Desipramine</td>
<td>Quinidine</td>
<td>0.5</td>
<td>$F_{ht}$ 0.62</td>
<td>$F_{ht}/F_{ht}$ 1.4</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>$F_{ht,1}$ 0.85</td>
<td>$CL_{ht}/CL_{ht,1}$ 4.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>$CL_{ht}$ 8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>$CL_{ht,1}$ 1.9</td>
</tr>
<tr>
<td>Desipramine</td>
<td>Ketoconazole</td>
<td>0.5</td>
<td>$F_{ht}$ 0.62</td>
<td>$F_{ht}/F_{ht}$ 1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$F_{ht,1}$ 0.63</td>
<td>$CL_{ht}/CL_{ht,1}$ 0.9</td>
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<td></td>
<td></td>
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<td>10</td>
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<td>0</td>
<td>$CL_{ht}$ 8.6</td>
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<td></td>
<td>0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>$CL_{ht,1}$ 9.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data for the reported drug-drug interactions were taken from the following literatures: terfenadine-ketoconazole,<sup>10,20</sup> indinavir-ketoconazole,<sup>21</sup> desipramine-quinidine<sup>22</sup> and desipramine-ketoconazole.<sup>23</sup>

<sup>b</sup> Substrate and inhibitor concentrations used in the serum incubation method were designated according to the reported and calculated values listed in Table 1.

<sup>c</sup> FH, hepatic availability in the absence of inhibitor; FH, I, hepatic availability in the presence of inhibitor; CLH, hepatic clearance in the absence of inhibitor (mL/min/kg); CLH, I, hepatic clearance in the presence of inhibitor (mL/min/kg).

<sup>d</sup> Steady-state systemic concentration of ketoconazole was achieved between 2 and 10 μM.<sup>10</sup>

<sup>e</sup> Reported value of maximum systemic plasma concentration of terfenadine (60-mg oral b.i.d.) was too low ($C_{\text{max}}$ = 0.003 μM) to be used for in vitro studies. Because the kinetics of terfenadine proceeded under linear condition between 0.02 and 0.2 μM as shown in Fig. 1, the same terfenadine concentration at 0.02 μM was used in the in vitro studies for the predictions of CLH in the presence or absence of ketoconazole.

<sup>f</sup> Because the effect of ketoconazole on the hepatic availability of indinavir was minimal (as shown by $F_{ht}/F_{ht}$ = 1.0–1.1), the same indinavir concentration at 2 μM was used in the in vitro studies for the predictions of CLH in the presence or absence of ketoconazole.

<sup>g</sup> Because the kinetics of desipramine proceeded under linear condition between 0.1 and 0.5 μM as shown in Fig. 3, the same desipramine concentration at 0.1 μM was used in the in vitro studies for the predictions of CLH in the presence or absence of quinidine.

<sup>h</sup> Steady-state systemic concentration of quinidine was achieved at around 1 μM.<sup>22</sup>

<sup>i</sup> Because the effect of ketoconazole on the hepatic availability of desipramine was minimal (as shown by $F_{ht}/F_{ht}$ = 1.02), the same desipramine concentration at 0.1 μM was used in the in vitro studies for the predictions of CLH in the presence or absence of ketoconazole.
Fig. 1. Inhibitory effect of ketoconazole on the metabolism of terfenadine in cryopreserved human hepatocytes as demonstrated by (A) disappearance curve of terfenadine, (B) in vitro clearance (CL_{int,serum}), (C) predicted hepatic availability (F_H) and (D) predicted hepatic clearance (CL_H). Initial metabolic rates of terfenadine at 0.02, 0.2 and 2 μM were measured in the presence of 0 (○), 0.04 (□), 0.2 (△), 0.6 (◇), 2.0 (■) and 10 (▲) μM ketoconazole at various time points (A). In vitro clearance (B), predicted hepatic availability (C) and predicted hepatic clearance (D) at the initial terfenadine concentrations of 0.02 (○), 0.2 (△) and 2.0 (■) μM were calculated along with the ketoconazole concentrations according to the method described in the section of Materials and Methods. Data for disappearance curves (A) represent the mean of three determinations. Data for in vitro clearance (B) were calculated from three individual disappearance curves of terfenadine based on Eq. (1) and are expressed as the mean ± SD. The values of F_H (C) and CL_H (D) were calculated from the mean values of in vitro clearance (B).

The values of hepatic plasma clearance (CL_H) and hepatic availability (F_H) were predicted from CL_{int,serum} based on the dispersion model \(^{36}\) as follows:

\[
CL_H = Q_H \times R_B \times \left( 1 - \frac{4a}{(1 + a)^2} \exp \left[ (a - 1)(2 \times D_N)\right] - (1 - a)^2 \exp \left[ - (a + 1)(2 \times D_N)\right] \right)
\]

\[
F_H = 1 - E_H = 1 - CL_H/(Q_H \times R_B)
\]

where \( R_N = (CL_{int,serum})/(Q_H \times R_B) \), \( a = (1 + 4 \times R_N \times D_N)^{0.5} \), \( Q_H \) = hepatic blood flow rate = 20.7 mL/min/kg, \(^{33}\) \( D_N \) = dispersion number = 0.17, \(^{36}\) and \( R_B \) represents blood-to-plasma concentration ratio.

The AUC after an oral administration (AUC_{po}) is expressed by the following equation, under the assumption...
Prediction of Drug-Drug Interaction in Humans

Fig. 2. Inhibitory effect of ketoconazole on the metabolism of indinavir in cryopreserved human hepatocytes as demonstrated by (A) disappearance curve of indinavir, (B) in vitro clearance (CL\text{int,serum}), (C) predicted hepatic availability (F\text{H}) and (D) predicted hepatic clearance (CL\text{H}). Initial metabolic rates of indinavir at 2.0 and 12 \text{mM} were measured in the presence of 0 (•), 0.4 (○), 2.0 (□), 10 (▲) and 50 (△) \text{μM} ketoconazole at various time points (A). In vitro clearance (B), predicted hepatic availability (C) and predicted hepatic clearance (D) at the initial indinavir concentrations of 2.0 (•) and 12 (△) \text{μM} were calculated along with the ketoconazole concentrations according to the method described in the section of Materials and Methods. Data for disappearance curves (A) represent the mean of three determinations. Data for in vitro clearance (B) were calculated from the AUC values of mean concentrations as described in the section of Materials and Methods. The values of F\text{H} (C) and CL\text{H} (D) were calculated from the mean values of in vitro clearance (B).

that the hepatic metabolism fully accounts for the elimination of substrate from body: AUC\text{po} = D × F\text{a} × F\text{H} × CL\text{H}

where F\text{a} and F\text{H} represent the fraction of absorption and hepatic availability, respectively. Therefore, the ratio of AUC in the presence of inhibitor (AUC\text{po,i}) to that in the absence of inhibitor (AUC\text{po}) is calculated from the following equation:

\[
\frac{\text{AUC}_{\text{po,i}}}{\text{AUC}_{\text{po}}} = \frac{\text{F}_{\text{H,i}}/\text{F}_{\text{H}} \times (\text{CL}_{\text{H,i}}/\text{CL}_{\text{H}}) \times (\text{F}_{\text{a,i}}/\text{F}_{\text{a}})}{\text{F}_{\text{a}}} = \text{F}_{\text{H,i}}.
\]

where F\text{H,i}, CL\text{H,i}, and F\text{a,i} represent hepatic availability, hepatic clearance and the fraction of absorption in the presence of inhibitor, respectively. In the present study, F\text{a} was assumed to be unaffected by the interaction (i.e. F\text{a} = F\text{a,i}).

Results

Terfenadine-ketoconazole interaction: Fig. 1A shows the disappearance curve of terfenadine at 0.02, 0.2 and 2 \text{μM} in the presence of 0, 0.04, 0.2, 0.6, 2 and 10 \text{μM} of ketoconazole in human hepatocytes suspended in 100% human serum. Terfenadine was log-linearly metabolized, and the initial disappearance rate constant at 2 \text{μM} terfenadine in the absence of ketoconazole was lower than that at 0.02 and 0.2 \text{μM}, suggesting that the saturation of metabolism occurred between 0.2 and 2 \text{μM} of terfenadine. Ketoconazole inhibited the metabolic
Fig. 3. Inhibitory effect of quinidine on the metabolism of desipramine in cryopreserved human hepatocytes as demonstrated by (A) disappearance curve of desipramine, (B) in vitro clearance (CL\textsubscript{int, serum}), (C) predicted hepatic availability (F\textsubscript{H}) and (D) predicted hepatic clearance (CL\textsubscript{H}). Initial metabolic rates of desipramine at 0.1 \textmu M and 0.5 \textmu M were measured in the presence of 0 (○), 0.01 (∇), 0.1 (△), 1.0 (□) and 10 (△) \textmu M quinidine at various time points (A). In vitro clearance (B), predicted hepatic availability (C) and predicted hepatic clearance (D) at the initial desipramine concentrations of 0.1 (○) and 0.5 (△) \textmu M were calculated along with the quinidine concentrations according to the method described in the section of Materials and Methods. Data for disappearance curves (A) represent the mean of three determinations. Data for in vitro clearance (B) were calculated from the AUC values of mean concentrations as described in the section of Materials and Methods. The values of F\textsubscript{H} (C) and CL\textsubscript{H} (D) were calculated from the mean values of in vitro clearance (B).

rate of terfenadine in a concentration-dependent manner. The values of in vitro clearance (CL\textsubscript{int, serum}) at different initial concentrations of terfenadine were calculated by Eq. (1) using the remaining terfenadine concentration at 120 min, and the values are plotted against ketoconazole concentration in Fig. 1B. The concentration of ketoconazole which 50% inhibited terfenadine metabolism (IC\textsubscript{50}) was about 1 \textmu M. By using the values of CL\textsubscript{int, serum} at various ketoconazole concentrations, clearance (CL\textsubscript{H}) and hepatic availability (F\textsubscript{H}) were calculated by Eqs. (2) and (3), respectively. The calculated values of F\textsubscript{H} and CL\textsubscript{H} are shown in Fig. 1C and Fig. 1D, respectively. Ketoconazole demonstrated potent inhibitory effects on both F\textsubscript{H} and CL\textsubscript{H} at higher than 0.6 \textmu M. The increase of terfenadine AUC after an oral administration by the interaction with ketoconazole was predicted by Eq. (4) using F\textsubscript{H}/F\textsubscript{H} and CL\textsubscript{H}/CL\textsubscript{H}. Results of calculation are summarized in Table 2. Predicted increase of terfenadine AUC by ketoconazole ranged from 6 to 37 fold, which was in a good agreement with the observed 13- to 59-fold increase of AUC in clinical studies.10,20)

Indinavir-ketoconazole interaction: Fig. 2A shows the disappearance curve of indinavir at 2 and 12 \textmu M in the presence of 0, 0.4, 2, 10 and 50 \textmu M of ketoconazole in human hepatocytes suspended in 100% human serum. In vitro clearance of indinavir at 12 \textmu M was less than
one-third of that at 2 μM in the absence of ketoconazole (Fig. 2B), suggesting that the saturation of indinavir metabolism occurred at < 12 μM. Ketoconazole inhibited the metabolic rate of indinavir in a concentration-dependent manner, and the value of IC₅₀ was approximately 10 μM at the initial indinavir concentration of 2 μM, whereas the inhibitory effect of ketoconazole was not clearly demonstrated at 12 μM (Fig. 2B). The increase of indinavir AUC after an oral administration by the interaction with ketoconazole was predicted by Eq. (4) using F₁₁/F₇₁ (Fig. 2C) and CL₁₁/CL₁₁ (Fig. 2D). Results of calculation are summarized in Table 2. Predicted increase of indinavir AUC by ketoconazole ranged from 1.4 to 2.0 fold, which was consistent with the minimal effect of ketoconazole on the AUC of indinavir (1.3-fold increase) reported from clinical study.²¹)

Desipramine-quinidine interaction: Fig. 3A shows the disappearance curve of desipramine at 0.1 and 0.5 μM in the presence of 0, 0.01, 0.1, 1 and 10 μM of quinidine in human hepatocytes suspended in 100% human serum. Quinidine inhibited metabolism of desipramine in a concentration-dependent manner as demonstrated by the decreases in both the initial disappearance rate constant (Fig. 3A) and the calculated in vitro clearance (Fig. 3B) along with the increase of quinidine concentration. Although the inhibitory effect of quinidine on the desipramine metabolism was very potent with an IC₅₀ value of approximately 0.1 μM (Fig. 3B), the predicted increase of desipramine F₁₁ was very small by a factor of 1.4 in the presence of 10 μM quinidine (Table 2 and Fig. 3C). On the other hand, the predicted decrease of CL₁₁ was large by a factor of 4.6-fold in the presence of 1 μM quinidine (Table 2 and Fig. 3D). The combination of predicted small increase in F₁₁ and large decrease in CL₁₁ resulted the overall prediction of 6.4-fold increase of desipramine AUC, which well agreed with the observed 6.7-fold increase of AUC reported from clinical drug-drug interaction study.²²)

Desipramine-ketoconazole interaction: Fig. 4 shows the effect of ketoconazole (0 and 10 μM) on the disappearance curves of desipramine at 0.1 and 0.5 μM in cryopreserved human hepatocytes suspended in 100% human serum. No inhibitory effect of ketoconazole on the desipramine metabolism was observed, which was consistent with the observation that ketoconazole treatment had little effect on the desipramine AUC reported from clinical study.²³)

Discussion

Based on the in vitro metabolic stability evaluated from serum incubation method using cryopreserved human hepatocytes suspended in 100% human serum, the increases in AUC of terfenadine, indinavir and desipramine were predicted in the drug-drug interactions with ketoconazole (for terfenadine, indinavir and desipramine) and quinidine (for desipramine) in humans. The serum incubation method well predicted these drug-drug interactions: the discrepancy between the increases of AUC predicted by the present method and those reported from clinical studies was within 50%, and in other word, the accuracy defined by the ratio of predicted to observed values ranged from 0.46 to 1.5. In addition, the accurate predictions obtained from the present study using separate calculations of drug-drug interactions during the first-pass metabolism (as predicted by the change of hepatic availability) and the elimination from systemic

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**Fig. 4.** Inhibitory effect of ketoconazole on the metabolism of desipramine in cryopreserved human hepatocytes as demonstrated by disappearance curve of desipramine. Initial metabolic rates of desipramine at 0.1 and 0.5 μM were measured in the presence of 0 (●) and 10 (○) μM ketoconazole. The values of in vitro clearance, predicted hepatic availability or predicted hepatic clearance were not calculated, since no inhibitory effect of ketoconazole on the metabolism of desipramine was observed. Data represent the mean of three determinations.
circulation (as predicted by the change of hepatic clearance) demonstrated that the substrate and inhibitor concentrations to be used in the in vitro experiments should be carefully designated based on the clinically relevant concentrations for the accurate predictions.

Terfenadine is an antihistamic drug and undergoes extensive first-pass metabolism mediated mainly by CYP3A4 in the liver after an oral administration. Clinical data indicated that terfenadine undergoes almost complete absorption and first-pass metabolism, suggesting that negligible contributions of urinary excretion and intestinal metabolism to the pharmacokinetics of terfenadine can be assumed for the calculation of drug-drug interaction by Eq. (4). Ketoconazole, one of the most potent and selective CYP3A4 inhibitors in theazole antifungal agents, has played a causative role in many drug-drug interactions. Therapeutic combination of terfenadine and ketoconazole resulted in the accumulation of terfenadine in patients, which was associated with life-threatening prolongation of QT interval. In the clinical study, steady-state was achieved for both terfenadine and ketoconazole in subjects taking terfenadine alone (60 mg b.i.d. for 7 days) or terfenadine with ketoconazole (200 mg b.i.d. for 7 days). Published data from clinical terfenadine-ketoconazole interaction study indicated that the values of steady-state AUC of terfenadine and ketoconazole [predicted increase of AUC of indinavir (Table 2)] suggested that the pharmacokinetics of indinavir for the hepatic elimination from systemic circulation is blood-flow-rate limited at clinically relevant concentration in humans. Both pharmacokinetics of indinavir in the absence of inhibitor [predicted AUC = 0.71 (Table 2) vs. observed AUC = 0.633) and predicted CLH = 16 ml/min/kg (Table 2) vs. observed CLH = 14.3–17.1 ml/min/kg] and marginal interaction between indinavir and ketoconazole [predicted increase of AUC of indinavir = 1.4–2.0 (Table 2) vs. observed increase of AUC = 1.32] were reasonably well predicted in the present study.

Desipramine, a tricyclic antidepressant, undergoes hepatic metabolism mediated by CYP2D6 as the major elimination pathway in humans. Clinical data indicated that desipramine was completely absorbed from intestine and the urinary excretion was minimal, suggesting that the elimination of desipramine is accounted for by the metabolism in the liver. Quinidine, an antiarrhythmic drug, is a potent competitive inhibitor for CYP2D6-mediated metabolism including desipramine 2-hydroxylation, and the Ki value of quinidine for the inhibition of desipramine 2-hydroxylation obtained from human liver microsomes (0.16 µM) was consistent with the IC50 value (0.1 µM) obtained in the present study. In the clinical study, the mean steady-state plasma concentration of quinidine was achieved at around 1 µM. The relatively small in vitro metabolic clearance for desipramine in the absence of inhibitor (0.85–0.91 µL/min/10⁶ cells in Fig. 3B) resulted in the prediction of high FGH value (0.62 in Table 2), which limits the inhibitory effect of quinidine on the first-pass metabolism of desipramine. On the other hand, the systemic clearance was predicted
to be metabolism-limited at clinically relevant concentration in humans with 8.6 ml/min/kg in the absence of inhibitor. Therefore, the drug-drug interaction by quindine was predicted to be more pronounced in the elimination process from systemic circulation (CLi/CLH = 4.6 in Table 2) than the process of first-pass metabolism (Fti/Fhu = 1.4 in Table 2).

The AUC value of desipramine following a single oral dose at 100 mg was little affected by the daily intake of 200 mg ketoconazole (4.8 μM·hr in control vs. 4.9 μM·hr with ketoconazole) in the clinical study. As for the large interaction between desipramine and quinidine, the minimal interaction between desipramine and ketoconazole in clinical study [observed 1.0-fold increase of AUC] was also well predicted by the present study [predicted 0.9-fold increase of AUC (Table 2)].

The present study clearly demonstrated that the in vivo inhibitory potency of inhibitor should highly depend on the inhibitor concentration which is also affected by its own pharmacokinetics: the maximum inhibitor concentration (as C_{in,abs}) can be used for the prediction of inhibitory potency to the substrate during the first-pass elimination, while the steady-state systemic concentration (as C_{s,max}) would be additionally used for the prediction of inhibition during the elimination of substrate from systemic circulation, as exemplified for all drug-drug interactions tested in this study. However, it should be also noted that the present method can still overestimate the predicted drug-drug interaction by assuming that the inhibitor concentrations remain constant over the time-course of substrate.

In summary, the “serum incubation method”, which had been used for the prediction of hepatic clearance and availability in humans, has proven to be a useful tool for the quantitative prediction of drug-drug interaction in humans. Separate experiments to examine potential factors affecting the accuracy of prediction are not necessary in the present method: the factors include plasma protein binding (and nonspecific microsomal binding), the optimal in vitro condition such as microsomal protein concentration, the potential involvement(s) of active transport(s) in the pharmacokinetics of substrate/inhibitor, and the metabolism by non-CYP drug metabolizing enzymes. In addition, the clinically relevant substrate and inhibitor concentrations can be directly designated to those used in the serum incubation method without additional interpretations or conversions of total to unbound concentrations. The method will provide useful background information on the design of clinical studies on drug-drug interaction based on the clinical data for the drug candidates become available from the first-in-man studies.

References


Prediction of Drug-Drug Interaction in Humans


