Physiologically Based Pharmacokinetic Model of Itraconazole and Two of Its Metabolites to Improve the Predictions and the Mechanistic Understanding of CYP3A4 Drug-Drug Interactions

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INTRODUCTION

Physiologically based pharmacokinetic (PBPK) modeling for itraconazole using a bottom-up approach is challenging, not only due to the complex saturable pharmacokinetics (PK) and the presence of three metabolites exhibiting CYP3A4 inhibition, but also because of discrepancies in reported in vitro data. The overall objective of this study is to provide a comprehensive mechanistic PBPK model for itraconazole in order to increase the confidence in its drug-drug interaction (DDI) predictions. To achieve this, key in vitro and in vivo data for itraconazole and its major metabolites were generated. These data were crucial to developing a novel bottom-up PBPK model in Simcyp (Simcyp Ltd., Certara, Sheffield, United Kingdom) for itraconazole and two of its major metabolites: hydroxy-itraconazole (OH-ITZ) and keto-itraconazole (keto-ITZ). Performance of the model was validated using prespecified acceptance criteria against different dosing regimens, formulations for 29 PK, and DDI studies with midazolam and other CYP3A4 substrates. The main outcome is an accurate PBPK model that simultaneously predicts the PK profiles of itraconazole, OH-ITZ, and keto-ITZ. In addition, itraconazole DDIs with midazolam and other CYP3A4 substrates were successfully predicted within a 2-fold error. Prediction precision and bias of DDI expressed as geometric mean fold error were for the area under the concentration-time curve and peak concentration, 1.06 and 0.96, respectively. To conclude, in this paper a comprehensive data set for itraconazole and its metabolites is provided that enables bottom-up mechanism-based PBPK modeling. The presented model is applicable for studying the contribution from the metabolites and allows improved assessments of itraconazole DDI.

ABBREVIATIONS: ACN, acetonitrile; AUC, area under the plasma concentration-time curve; CL, clearance; CLR, renal clearance; DDI, drug-drug interaction; DMSO, dimethylsulfoxide; EAC, enzyme activity change; f1, difference factor; fU,MIC, fraction of unbound drug in microsomal incubation; fU,Pr, fraction of unbound drug in plasma; GMFE, geometric mean fold error; HLM, human liver microsomes; keto-ITZ, keto-itraconazole; KI, constant of inhibition; LCI, Michaelis constant; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MDCM, multiple depletions curve method; ND-ITZ, N-desalkyl itraconazole; NLME, nonlinear mixed effect; OH-ITZ, hydroxy-itraconazole; PK, pharmacokinetics; SAC, single adjustable compartment; SI, sensitivity index; Vmax, theoretical maximum depletion rate; Vss, volume of distribution.

1420
identifiability and model misspecification. The middle-out approach is a combination of the other two.

Several challenges hinder the development of PBPK models for itraconazole. First, the complex PK with saturable CYP3A4 metabolism and inhibition presents a challenge. All three metabolites are potent CYP3A4 inhibitors and their contribution to clinical DDIs has been considered relevant in a first assessment using static equations (Templeton et al., 2008). Second, there is a lack of robust in vitro data in the literature, which causes uncertainty when building a mechanistic PBPK model based on a bottom-up approach (Chen et al., 2016). Large variabilities in reported in vitro data are mainly due to solubility issues and high protein binding for itraconazole itself as well as its metabolites (Heykants et al., 1989; Isoherranen et al., 2004). The existing itraconazole PBPK library model in Simcyp includes only one metabolite (OH-ITZ) and tends to overpredict the DDI risk (Marsoussi et al., 2018). Although Chen et al. (2016) recently published a PBPK model for itraconazole and OH-ITZ using a top-down approach to overcome the challenges of obtaining robust in vitro data from the literature, it is important to continue the efforts of producing robust in vitro data to further develop a more mechanistic PBPK model and this has been emphasized previously for itraconazole (Chen et al., 2016). We believe that further improvements to existing PBPK models are needed, ideally including all metabolites to assess in depth their contribution to the DDI. One limitation with the software selected for our PBPK model is that it is only possible to have two metabolites included. Given the sequential formation of the metabolites, OH-ITZ and keto-ITZ were included in our model and in vitro data for ND-ITZ was generated to be incorporated in future models.

The overall objective of this study is to provide a comprehensive mechanistic PBPK model for itraconazole to increase the confidence in its DDI predictions. To achieve this, key in vitro and in vivo data for itraconazole and its major metabolites were generated, providing improved PK knowledge. These new data were crucial to the development of a bottom-up PBPK model for itraconazole incorporating for first time two of its major metabolites: OH-ITZ and keto-ITZ. Thus, in this paper we provide a comprehensive data set for itraconazole and its three metabolites. This data set enables the development of a bottom-up mechanistic PBPK model that is applicable of assessing clinical itraconazole DDIs and studying the contribution from its metabolites to the DDI.

Materials and Methods

Clinical Data Collection

A total of 11 clinical studies, previously collected by Chen et al. (2016), were used for PK profile verification of itraconazole and OH-ITZ. Two studies included data for keto-ITZ and were used for model verification of this metabolite (Templeton et al., 2008; Liang et al., 2016). Plasma concentration-time profiles and variability were extracted from the figures in the publications. A total of 18 DDI studies using itraconazole as a CYP3A4 inhibitor were used for model validation. Ten clinical DDI studies including midazolam as the substrate have been reported and previously collected by Chen et al. (2016). Eight DDI studies involving other CYP3A4 substrates previously collected by Marsoussi et al. (2018) were also used. Different dosing regimens, such as single or multiple dosing with oral solution or capsule formulation of itraconazole were given in these studies. Detailed clinical trial information including original references and the simulated trial designs are described in Tables 1 and 2.

Table 1

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Number</th>
<th>Route</th>
<th>Dose (mg)</th>
<th>Regimen</th>
<th>Itraconazole Formulation</th>
<th>Fasted/Fed</th>
<th>PK Data Used in PBPK model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Oral</td>
<td>100</td>
<td>Once daily x 7 days</td>
<td>Solution</td>
<td>Fasted</td>
<td>Development; verification</td>
<td>Templeton et al. (2008)</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Oral</td>
<td>200</td>
<td>Once daily x 11 days</td>
<td>Solution</td>
<td>Fasted</td>
<td>Verification</td>
<td>Liang et al. (2016)</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>Oral</td>
<td>200</td>
<td>Once daily x 15 days</td>
<td>Solution</td>
<td>Fed/Fasted</td>
<td>Verification</td>
<td>Barone et al. (1998)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>Oral</td>
<td>100</td>
<td>Once daily x 15 days</td>
<td>Capsule</td>
<td>Fed</td>
<td>Verification</td>
<td>Van Peer et al. (1989)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Oral</td>
<td>100</td>
<td>Once daily x 15 days</td>
<td>Capsule</td>
<td>Fed</td>
<td>Verification</td>
<td>Hardin et al. (1988)</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Oral</td>
<td>100</td>
<td>Once daily x 4 days</td>
<td>Capsule</td>
<td>Fed</td>
<td>Verification</td>
<td>Heykants et al. (1989)</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Oral</td>
<td>200</td>
<td>Once daily x 4 days</td>
<td>Capsule</td>
<td>Fasted</td>
<td>Verification</td>
<td>Ohkubo and Otsuka (2005)</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Oral</td>
<td>200</td>
<td>Once daily x 6 days</td>
<td>Capsule</td>
<td>Fasted</td>
<td>Verification</td>
<td>Uno et al. (2006)</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>Oral</td>
<td>200</td>
<td>Once daily x 10 days</td>
<td>Capsule</td>
<td>Fasted</td>
<td>Verification</td>
<td>Miura et al. (2010)</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>Oral</td>
<td>200</td>
<td>Twice daily x 15 days</td>
<td>Capsule</td>
<td>Fed</td>
<td>Verification</td>
<td>Barone et al. (1993)</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>Oral</td>
<td>200</td>
<td>Twice daily x 15 days</td>
<td>Capsule</td>
<td>Fed</td>
<td>Verification</td>
<td>Hardin et al. (1988)</td>
</tr>
</tbody>
</table>

* Refers to the number of healthy volunteers in the clinical study reported.
### TABLE 2
Comparison between predicted and observed clinical DDI studies

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Itraconazole Dose Regimen</th>
<th>CYP3A4 Substrate</th>
<th>Predicted DDI Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Observed DDI Ratio</th>
<th>Model Validation Predicted/Observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>200 mg once daily x 4 days, capsule</td>
<td>Midazolam 7.5 mg, day 4, 1 h post-ITZ</td>
<td>8.9</td>
<td>10.8</td>
<td>0.8</td>
<td>Templeton et al. (2010)</td>
</tr>
<tr>
<td>13</td>
<td>100 mg once daily x 4 days, capsule</td>
<td>Midazolam 7.5 mg, day 4, 2 h post-ITZ</td>
<td>5.0</td>
<td>5.7</td>
<td>0.9</td>
<td>Templeton et al. (2010)</td>
</tr>
<tr>
<td>14</td>
<td>200 mg once daily x 1 day, capsule</td>
<td>Midazolam 7.5 mg, day 1, 2 h post-ITZ</td>
<td>6.4</td>
<td>3.4</td>
<td>1.9</td>
<td>Templeton et al. (2010)</td>
</tr>
<tr>
<td>15</td>
<td>200 mg once daily x 4 days, capsule</td>
<td>Midazolam 0.05 mg, IV, day 4, 2 h post-ITZ</td>
<td>3.6</td>
<td>3.2</td>
<td>1.1</td>
<td>Olkkola et al. (1996)</td>
</tr>
<tr>
<td>16</td>
<td>200 mg once daily x 6 days, capsule</td>
<td>Midazolam 7.5 mg, day 6, 2 h post-ITZ</td>
<td>8.6</td>
<td>6.6</td>
<td>1.3</td>
<td>Olkkola et al. (1996)</td>
</tr>
<tr>
<td>17</td>
<td>200 mg once daily x 4 days, capsule</td>
<td>Midazolam 7.5 mg, day 4, 2 h post-ITZ</td>
<td>7.6</td>
<td>8.0</td>
<td>0.9</td>
<td>Alhonen et al. (1995)</td>
</tr>
<tr>
<td>18</td>
<td>200 mg once daily x 4 days, capsule</td>
<td>Midazolam 7.5 mg, day 8, 4 days post-ITZ&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3</td>
<td>2.6</td>
<td>0.5</td>
<td>Olkkola et al. (1996)</td>
</tr>
<tr>
<td>19</td>
<td>50 mg single dose, solution</td>
<td>Midazolam 2 mg, 4 h post-ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.5</td>
<td>2.0</td>
<td>1.2</td>
<td>Backman et al. (1998)</td>
</tr>
<tr>
<td>20</td>
<td>200 mg single dose, solution</td>
<td>Midazolam 2 mg, 4 h post-ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.8</td>
<td>4.7</td>
<td>1.4</td>
<td>Backman et al. (1998)</td>
</tr>
<tr>
<td>21</td>
<td>400 mg single dose, solution</td>
<td>Midazolam 2 mg, 4 h post-ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.7</td>
<td>5.4</td>
<td>2.0</td>
<td>Olkkola et al. (1994)</td>
</tr>
<tr>
<td>22</td>
<td>200 mg once daily x 4 days, capsule</td>
<td>Simvastatin 40 mg, day 4, 2 h post-ITZ</td>
<td>18.4</td>
<td>17</td>
<td>1.0</td>
<td>Neuvonen et al. (1998)</td>
</tr>
<tr>
<td>23</td>
<td>200 mg once daily x 4 days, capsule</td>
<td>Triazolam 0.25 mg, day 4, 1 h post-ITZ</td>
<td>8.6</td>
<td>19&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.5</td>
<td>Varhe et al. (1994)</td>
</tr>
<tr>
<td>24</td>
<td>200 mg single dose, capsule</td>
<td>Triazolam 0.25 mg, simultaneously with ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.2</td>
<td>3.1</td>
<td>2.3</td>
<td>Neuvonen et al. (1996)</td>
</tr>
<tr>
<td>25</td>
<td>200 mg single dose, capsule</td>
<td>Triazolam 0.25 mg, 3 h post-ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.5</td>
<td>4.5</td>
<td>1.2</td>
<td>Neuvonen et al. (1996)</td>
</tr>
<tr>
<td>26</td>
<td>200 mg single dose, capsule</td>
<td>Triazolam 0.25 mg, 12 h post-ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.9</td>
<td>4.3</td>
<td>0.7</td>
<td>Neuvonen et al. (1996)</td>
</tr>
<tr>
<td>27</td>
<td>200 mg single dose, capsule</td>
<td>Triazolam 0.25 mg, 24 h post-ITZ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.0</td>
<td>3.8</td>
<td>0.5</td>
<td>Neuvonen et al. (1996)</td>
</tr>
<tr>
<td>28</td>
<td>200 mg once daily x 6 days, capsule</td>
<td>Alprazolam 0.8 mg, day 4, 1 h post-ITZ</td>
<td>2.4</td>
<td>2.7</td>
<td>0.9</td>
<td>Yasui et al. (1998)</td>
</tr>
<tr>
<td>29</td>
<td>100 mg once daily x 4 days, capsule</td>
<td>Quinidine 100 mg, day 4, 1 h post-ITZ</td>
<td>3.5</td>
<td>1.6</td>
<td>1.4</td>
<td>Kaukonen et al. (1997)</td>
</tr>
</tbody>
</table>

<sup>a</sup>ITZ, itraconazole.
<sup>b</sup>GMFE = 1.06
<sup>c</sup>All predicted AUC ratios are presented as the geometric mean.
<sup>d</sup>The AUC and C<sub>max</sub> ratios presented are for the CYP3A4 substrate with and without the presence of itraconazole.
<sup>e</sup>AUC<sub>ITZ</sub> and C<sub>max,ITZ</sub> refer to the ratio between predicted vs. observed AUC or C<sub>max</sub> ratios.
<sup>f</sup>Evaluation after 4-day washout of itraconazole.
<sup>g</sup>Evaluation after a single dose of itraconazole.

ITZ, itraconazole.

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<sup>b</sup>ITZ, itraconazole.
<sup>c</sup>All predicted AUC ratios are presented as the geometric mean.
<sup>d</sup>The AUC and C<sub>max</sub> ratios presented are for the CYP3A4 substrate with and without the presence of itraconazole.
<sup>e</sup>AUC<sub>ITZ</sub> and C<sub>max,ITZ</sub> refer to the ratio between predicted vs. observed AUC or C<sub>max</sub> ratios.
<sup>f</sup>Evaluation after 4-day washout of itraconazole.
<sup>g</sup>Evaluation after a single dose of itraconazole.
<sup>h</sup>The AUC and C<sub>max</sub> ratios were calculated from the published data.
Substrate Depletion Experiment. A substrate depletion method was used to identify the enzyme kinetic parameters: the maximum velocity of the metabolic reaction (V_{\text{max}}) and the Michaelis constant (K_{m}) of itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ. Itraconazole and its metabolites were incubated at 37°C 600 rpm with recombinant CYP3A4 enzymes (15 nM). All experiments were optimized for protein and time to ensure linearity and run at different test occasions (n = 3). Substrates were added to incubations from a 50 mM stock solution in 90% ACN. Nominal substrate concentrations in the final incubation were 5, 10, 20, 40, 80, 160, 320, and 500 nM and the total ACN content in the incubation mixture did not exceed 0.1%. After preincubation for 5 minutes, the incubation was initiated with NADPH to a final concentration of 1 mM. At time points 1, 2, 3.5, 5, 10, and 15 minutes, 15 μl samples were quenched with 30 μl of ice-cold ACN. Then, samples were centrifuged (3220g at 4°C for 30 minutes) and supernatant was transferred and diluted for analysis by LC-MS/MS.

Calculation of Enzyme Kinetics Parameters. The K_{m} and V_{\text{max}} values were determined according to the multiple depletions curve method (MDCM) described by Sjögren et al. (2009). The model including enzyme activity change (EAC) was selected (eq. 1). The variable EAC was described as monoexponential decay (MDCM + EAC constant), taking into account potential enzyme degradation and/or inhibition effects during the incubation period: 

\[ \frac{d[C]}{dt} = \frac{V_{\text{max}} - [C]}{K_{m} + [C]} \times e^{-k_{E}t} \]  

(eq. 1)

where V_{\text{max}} is the theoretical maximum depletion rate; K_{m} is the substrate concentration at one-half the V_{\text{max}}; [C] is the substrate concentration; and k_{E} is the EAC constant. The parameters, v_{\text{max}} and K_{m}, were estimated by simultaneous fitting of the equation to all concentration-time profiles using the nonlinear mixed effect (NLME) in Phoenix NLME 15 version 6.0 (Pharsight Corporation, A Certara Company, Princeton, NJ). The V_{\text{max}} value was obtained by dividing v_{\text{max}} by the protein concentration used in the incubation (C_{P}):

\[ V_{\text{max}} = \frac{v_{\text{max}}}{C_{P}} \]  

(eq. 2)

CYP3A4 Inhibition Assay. The inhibition parameter describing the inhibitor concentration that reduces the enzyme activity by 50% (the IC_{50} value) for itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ in HLM was measured using 1’-hydroxylation of midazolam as a probe reaction for CYP3A4-mediated metabolism. The incubations were performed with 0.2 mg/ml pooled HLM in 100 mM phosphate buffer (pH 7.4) with 1 mM EDTA and a total concentration of 1 mM NADPH (n = 3). The midazolam concentration was 3 μM. The optimal substrate conditions were previously internally validated. Itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ were added at nominal concentrations of 0, 3, 10, 30, 100, 300, and 1000 nM. The final concentration of DMSO:ACN was 0.3:0.7% v/v. A time-zero sample was collected after preincubation for 5 minutes at 37°C and the reaction was initiated by the addition of NADPH. A second sample was taken after a 5-minute incubation. All samples were quenched with ice-cold ACN (1:1). Then, the samples were centrifuged (3220g for 30 minutes) and supernatant was transferred and diluted for analysis by LC-MS/MS.

The IC_{50} value was determined by fitting the experimental data to an I_{\text{max}} model (eq. 3) using Phoenix NLME:

\[ E = E_{0} \times \left(1 - \frac{C}{IC_{50} + C}\right) \]  

(eq. 3)

where E is the effect of inhibition; E_{0} is the baseline; and C is the concentration of substrate. The constant of inhibition (K_{I}) was calculated by a classical competitive inhibition model described in eq. 4:

\[ K_{I} = \frac{IC_{50}}{[S/K_{m}(\text{in situ})] + 1} \]  

(eq. 4)

where S is the concentration in the incubation and K_{m}(\text{in situ}) is the Michalis constant of midazolam (2 μM) (Cer et al., 2009).

Pharmacokinetic Study of Intravenous Administration in Rat. Male Han Wistar rats (n = 2 – 300 g) (Charles River, Sulzfeld, Germany), were dosed with keto-ITZ solution (1 mg/kg, 1 ml/kg, bolus) intravenously to the tail vein. The formulation was 5% DMSO and 95% 2-hydroxypropyl-beta-cyclodextrin (30% w/v) in water adjusted to pH 4. Blood samples were collected into EDTA-coated tubes at 2, 7, 15, 30, 60, 120, 240, 360, 420, and 1440 minutes after dose. Urine samples were also collected during the following intervals 0–120, 120–360, and 360–1440 minutes after dose. All samples were centrifuged at 3220g for 5 minutes at 4°C. Blood samples were immediately stored in polypropylene tubes at −80°C and deproteinized by solvent precipitation prior to analysis. Samples were diluted for analysis by LC-MS/MS. The PK parameters were estimated by one-compartmental analyses using Phoenix NLME.

Before the study, the animals were acclimatized for a minimum of 5 days and allowed food and water ad libitum. All of the animal studies were conducted in accordance with the National Institutes of Health guidelines on animal welfare.

Model Acceptance Criterion for PK Verification and DDI Validation

The following criteria were predetermined to assess model performance. First, the performance of the model in describing the PK profiles of itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ was verified if the observed concentration-time profiles were within the 90% prediction interval (5th to 95th percentile range of the virtual population). Following the verification of the PK model for itraconazole and its metabolites, simulations were performed to test the capability of the model to accurately describe DDI studies with itraconazole and midazolam. The geometric mean area under the plasma concentration-time curve (AUC) ratio for each DDI study was determined and the predicted and observed data were compared (see Table 2 for results). Precision and bias for the DDI predictions were evaluated using the geometric mean fold error (GMFE) described in eq. 5:

\[ \text{GMFE} = \frac{10^{\ln(\text{Predited DDI/Oberved DDI})}}{\text{Number of predictions}} \]  

(eq. 5)
## TABLE 3
Input drug-dependent parameter values used for itraconazole and its metabolites in the PBPK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Itraconazole</th>
<th>OH-Itraconazole</th>
<th>Keto-Itraconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW (g/mol)</td>
<td>705.6</td>
<td>Library V15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>721.6</td>
</tr>
<tr>
<td>Log&lt;sub&gt;P&lt;sub&gt;ow&lt;/sub&gt;&lt;/sub&gt;</td>
<td>4.47</td>
<td>Library V15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.64</td>
</tr>
<tr>
<td>p&lt;sub&gt;k&lt;/sub&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>4.28</td>
<td>Library V15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.28</td>
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<tr>
<td>B/P</td>
<td>0.58</td>
<td>Library V15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58</td>
</tr>
<tr>
<td>&lt;sup&gt;fu&lt;/sup&gt;&lt;sub&gt;p&lt;/sub&gt;</td>
<td>0.01</td>
<td>FDA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.012</td>
</tr>
<tr>
<td>Absorption model</td>
<td></td>
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<td></td>
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<tr>
<td>&lt;sub&gt;p&lt;/sub&gt;&lt;sub&gt;_a&lt;/sub&gt;</td>
<td>1/0.59</td>
<td>SolCap Library V15&lt;sup&gt;a&lt;/sup&gt; (first order)</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;a&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.5/0.6</td>
<td>SolCap Library V15&lt;sup&gt;a&lt;/sup&gt; (first order)</td>
<td></td>
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<tr>
<td>Q&lt;sub&gt;out&lt;/sub&gt; (l/h)</td>
<td>57.1</td>
<td>Library V15&lt;sup&gt;a&lt;/sup&gt; (first order)</td>
<td>15.7</td>
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<td>Distribution model</td>
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<tr>
<td>V&lt;sub&gt;u&lt;/sub&gt; (l/Kg)</td>
<td>3.9</td>
<td>Mouton et al. (2006) (full PBPK)</td>
<td>3.8</td>
</tr>
<tr>
<td>V&lt;sub&gt;u&lt;/sub&gt;&lt;sub&gt;rat&lt;/sub&gt; (Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k&lt;sub&gt;in&lt;/sub&gt;/k&lt;sub&gt;out&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elimination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; CYP3A4 (pmol/min per micromole isofrom)</td>
<td>5.31</td>
<td>Internally measured&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.66</td>
</tr>
<tr>
<td>K&lt;sub&gt;a&lt;/sub&gt; CYP3A4 (µM)</td>
<td>0.28</td>
<td>Internally measured&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.11</td>
</tr>
<tr>
<td>System (ISEF)</td>
<td>0.19</td>
<td>Internally measured&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.65</td>
</tr>
<tr>
<td>C&lt;sub&gt;L&lt;/sub&gt;&lt;sub&gt;rat&lt;/sub&gt; (l/h)</td>
<td>0.25</td>
<td>Cypex HR</td>
<td>2.25</td>
</tr>
<tr>
<td>Hepatic uptake</td>
<td>0.25</td>
<td>Cypex HR</td>
<td>0.39</td>
</tr>
<tr>
<td>Cytochrome P450 inhibition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;c&lt;/sub&gt; CYP3A4 (µM)</td>
<td>0.038</td>
<td>Internally measured&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.042</td>
</tr>
<tr>
<td>&lt;sup&gt;fu&lt;/sup&gt;&lt;sub&gt;miz&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.01</td>
<td>Internally measured&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.076</td>
</tr>
</tbody>
</table>

B/P, blood-to-plasma ratio; <sub>fu</sub>, fraction of dose absorbed; FDA, US Food and Drug Administration; <sub>p<sub>_a</sub></sub>, fraction of unbound drug in the gut; HR, high reductase; ISEF, inter system extrapolation factor for scaling of recombinant CYP in vitro kinetic data; <sub>k</sub><sub>_a</sub>, absorption constant; <sub>k</sub><sub>in</sub>, first-order rate constant for the distribution to a single adjustable compartment; <sub>k</sub><sub>out</sub>, first-order rate constant for the distribution from a single adjustable compartment; MW, molecular weight; Q<sub>out</sub>, nominal flow through the gut; <sub>V<sub>SS</sub></sub> SAC, SAC volume.

<sup>a</sup>Refers to the Simcyp simulator compound library (version 15).

<sup>b</sup>Determined using software Biobyte (Pomona College and BioByte, Inc.).

<sup>c</sup>All compounds are monoprotic base (Simcyp Library version 15).

<sup>d</sup>The US Food and Drug Administration recommends that of <sub>fu</sub> should be set to 0.01 if experimentally determined to be <0.01 (US Food and Drug Administration Guidance for In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm).

<sup>e</sup>Refers to data published in this paper, details in Methods and Materials, and values to Table 4.

<sup>f</sup>The <sub>V<sub>u</sub></sub> value was scaled from rat to human to human using the the Oie-Tozer method (Oie and Tozer, 1979). Different rat <sub>V<sub>u</sub></sub> references were used for OH-ITZ (Yoo et al., 2000) and keto-ITZ in-house data (Table 4).

<sup>g</sup>SAC parameters were optimized to match PK profiles at day 1 of data set 1.

<sup>h</sup>Calculated from internally measured <sub>f<sub>u</sub></sub><sub>miz</sub> and corrected by protein concentration in the assay (Wan and Rehngren, 2006).
The hepatic CL for itraconazole and its metabolites was scaled in the model from the CYP3A4 enzyme kinetic parameters $K_{m}$ and $V_{max}$ that were determined by the MDCM method described previously. The renal CL (CLR) for keto-ITZ was scaled from rat using the Simcyp toolbox. In the case of keto-ITZ, CYP3A4 metabolism and CLR were not sufficient to describe the observed CL in humans. In addition, unspecific CL was included for this compound to capture a potential alternative route of elimination. The additional CL was added using the retrograde method and the CL determined in rats and scaled to humans was used as the starting value.

The $f_{mic}$ determined by dialysis in microsomal incubation mixtures was incorporated into each model to correct for protein binding in the in vitro experiments. The $f_{mic}$ value was corrected for the protein concentrations used in each assay (Table 3). Specifically, for enzyme kinetics parameters ($f_{mic1}$), the method described by Wan and Rehngren (2006) was used. For inhibition parameters ($f_{mic2}$), where protein concentration was similar to measured $f_{mic}$ conditions, no correction was deemed necessary based on the binding profile of OH-ITZ (Tran et al., 2002).

**Simulations Using PBPK Modeling**

The Simcyp (version 15) population-based PBPK simulator (Simcyp Ltd., Certara) was used to simulate the PK of itraconazole and its metabolites (OH-ITZ and keto-ITZ), and relevant DDI in virtual healthy volunteers. Simulations were performed with randomly selected individuals from a simulated healthy volunteer population built in the Simcyp software (Sim-Healthy). A total of 100 individuals—10 trials with 10 individuals per trial—were simulated to assess variability across groups. The age, sex ratio, dose, formulation, and regimen used in the simulations were matched to the clinical studies described in each trial (Tables 1 and 2). The CYP3A4 substrate models available in the Simcyp compound library were directly used in the simulations. When the DDI simulations were performed, the itraconazole compound file including the two metabolites were put as the substrate file and output was exported to Phoenix. The AUCs from each simulation were calculated by noncompartmental analyses and the log/linear trapezoidal method. The relative contribution of itraconazole and its metabolites (OH-ITZ and keto-ITZ) to the total AUC ratio was calculated following the approach of Wang et al. (2004), where net inhibition is related to the sum of inhibitory contributions of all circulating inhibitors as described in Templeton et al. (2008). Simulated unbound liver and gut concentrations and the respective apparent $K_{i,u}$ for itraconazole and its metabolites were used in the calculation. The simulated concentrations were taken from a dosing regimen of once daily administration for 4 days of 200 mg itraconazole (capsule and oral solution) given in the fasted state.

**Model Parameter Sensitivity Analysis**

Sensitivity analysis in this context is a description of how sensitive the model is to changes in the model parameters. Key parameters were evaluated in this sensitivity analysis to gain additional insight on their impact on the AUC of itraconazole and its metabolites. The sensitivity analysis was done using two different methods: The one-factor-at-a-time approach and the sensitivity index (SI) approach (Nestorov, 1999; Bonate, 2011). In the one-factor-at-a-time approach, the numerical value of a parameter is varied within a specified region around the estimated optimal parameter value while the change in AUC is observed. The SI for each parameter was calculated using eq. 6:

$$SI = \frac{AUC_{max} - AUC_{min}}{AUC_{max}}$$

where $AUC_{max}$ and $AUC_{min}$ are the maximum and minimum AUCs, respectively, within the explored parameter space in the sensitivity analysis. The advantage of the SI compared with the one-factor-at-a-time methods is that a direct comparison of the estimated sensitivity of the model parameters is possible.

**Postanalysis of the Simulated Plasma Profiles**

To further assess the performance of the model on simulating PK profiles in a quantitative manner, a postanalysis was conducted according to previously established methods (Marston and Polli, 1997). The difference factor ($f_{1}$), which is a model-independent parameter, was applied for the comparison of the plasma concentration-time profiles of itraconazole, OH-ITZ, and keto-ITZ according to eq. 7:

$$f = \frac{\sum \left| R_t - T_t \right|}{\sum R_t} \cdot 100$$

where $n$ is the number of time points, and $R_t$ and $T_t$ are the plasma drug concentrations observed and simulated, respectively, at each time point $t$. 

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**Fig. 1.** Workflow of the development, verification, and validation of the PBPK model for itraconazole, OH-ITZ, and keto-ITZ.
intravenous was bound to plasma proteins in human and rat animals (Supplemental Fig. 2) and the PK parameters (Fig. 3A). In addition, the simulated IC50 curves with remaining enzyme depletion or inhibition. All four compounds had low unbound parameters is presented in Table 4. All concentration-time profiles and data set in a step-wise manner. The simulated plasma concentration was first developed and optimized using data set 1 (Table 1) as a training results from in-vitro and in-vivo experiments

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Itraconazole</th>
<th>OH-ITZ</th>
<th>Keto-ITZ</th>
<th>ND-ITZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human PPB (% free)</td>
<td>0.1 (22%)</td>
<td>1.2 (60%)</td>
<td>1 (39%)</td>
<td>2.9 (23%)</td>
</tr>
<tr>
<td>Iunmic</td>
<td>0.01 (10%)</td>
<td>0.076 (10%)</td>
<td>0.063 (10%)</td>
<td>0.066 (10%)</td>
</tr>
<tr>
<td>Km, CYP3A4 (μM)</td>
<td>0.28 (30%)</td>
<td>0.11 (22%)</td>
<td>0.08 (20%)</td>
<td>0.05 (17%)</td>
</tr>
<tr>
<td>Vmax, CYP3A4 (pmol/min per micromoles of isofom)</td>
<td>5.31 (26%)</td>
<td>1.6 (17%)</td>
<td>1.84 (16%)</td>
<td>0.99 (12%)</td>
</tr>
<tr>
<td>kel (min⁻¹)</td>
<td>0.09 (12%)</td>
<td>0.08 (16%)</td>
<td>0.11 (12%)</td>
<td>0.05 (21%)</td>
</tr>
<tr>
<td>IC50 (μM)</td>
<td>0.1 (3%)</td>
<td>0.111 (4%)</td>
<td>0.098 (9%)</td>
<td>0.136 (5%)</td>
</tr>
<tr>
<td>Apparent Ki (μM)</td>
<td>0.038</td>
<td>0.042</td>
<td>0.04</td>
<td>0.051</td>
</tr>
<tr>
<td>Apparent KM (μM)</td>
<td>0.0004</td>
<td>0.0032</td>
<td>0.0025</td>
<td>0.0034</td>
</tr>
<tr>
<td>Rat Vc (l/kg)</td>
<td>—</td>
<td>—</td>
<td>3.16 (24%)</td>
<td>—</td>
</tr>
<tr>
<td>Rat CLiv (ml/min per kilogram)</td>
<td>—</td>
<td>—</td>
<td>79.78 (11%)</td>
<td>—</td>
</tr>
<tr>
<td>Rat CLm (ml/min per kilogram)</td>
<td>—</td>
<td>—</td>
<td>0.04 (18%)</td>
<td>—</td>
</tr>
<tr>
<td>Rat PPB (% free)</td>
<td>—</td>
<td>0.61 (17%)</td>
<td>0.43 (18%)</td>
<td>—</td>
</tr>
</tbody>
</table>

k_e, enzyme activity change constant; PPB, plasma protein binding. — , not measured.

*All data presented as arithmetic mean and CV (%) (n = 3).

Calculated by eq. 4 (Cer et al., 2009).

Calculated by correcting with the measured Iunmic.

These data represent the arithmetic mean of two animals.

Results

Experimental In Vitro Determination

Human and Rat Plasma Protein Binding. Human and rat plasma protein binding was assessed using equilibrium dialysis. All test compounds were highly bound to plasma proteins in human and rat resulting in low Iunmic values (0.001–0.029), which are presented in Table 4.

Fraction Unbound in HLM Incubations. Protein binding was also determined in HLM incubation mixtures by dialysis for itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ. All of the test compounds were extensively bound and this resulted in overall low Iunmic values (0.01–0.076). The details are presented in Table 4.

Enzyme Kinetics Analysis. The CYP3A4 enzyme kinetic parameters (Vmax and Km) for itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ were estimated using the MDCM method. A summary of the resulting parameters is presented in Table 4. All concentration-time profiles and model fits for itraconazole and its three metabolites are presented in Fig. 2. Biphasic depletion curves were observed for all four substrates, and therefore MDCM with EAC was used to take into account potential enzyme depletion or inhibition. All four compounds had low unbound Km values, indicating high affinity to CYP3A4.

CYP3A4 Inhibition. The potency of the CYP3A4 inhibition for itraconazole, OH-ITZ, keto-ITZ, and ND-ITZ was determined in HLM using midazolam as the substrate. A summary of the resulting parameters (IC50 and Ki) is presented in Table 4. The fitted IC50 curves versus a range of inhibitor concentrations are presented in Fig. 3A. In addition, the simulated IC50 curves with remaining midazolam (%) versus calculated free concentrations are presented in Fig. 3B.

Rat PK. Rats were intravenously dosed with keto-ITZ with the purpose of obtaining Ve, CL, and CLR for this metabolite. The plasma and urine concentration-time curves for the individual animals (Supplemental Fig. 2) and the PK parameters (Ve, CL, and CL-R) calculated by noncompartmental analysis are presented in Table 4.

PBPK Model Development and Verification

The PBPK model for itraconazole, OH-ITZ, and keto-ITZ (Table 3) was first developed and optimized using data set 1 (Table 1) as a training data set in a step-wise manner. The simulated plasma concentration profiles of the training data set met the predefined model acceptance criteria (observed plasma concentration falling within the 90% prediction interval) (Fig. 4, A, C, and E). Furthermore, the model was verified using a separate test data set of 10 human PK studies including observations at a wide range of time points varying from 1 day up to 4 weeks across a multiple dosings regimen, both for solution and capsule formulation at different doses, and under different fed and fasted conditions. Similar to the training data set, all of the simulated concentration profiles (Fig. 4, B, D, and F; Fig. 6) for the verification studies fell within the acceptance criteria. Accumulation over days was reasonably well captured by the model for all three compounds under all of the different conditions (Figs. 4 and 5).

Model Parameter Sensitivity Analysis

To gain additional insights into this complex model of a parent compound and two sequential metabolites in which all are substrates and inhibitors of CYP3A4, a sensitivity analysis was conducted to assess the relative importance of the key parameters toward changes in itraconazole, OH-ITZ, and keto-ITZ AUC. In general, the AUCs for all three compounds are more sensitive to elimination kinetics parameters and plasma protein binding than to inhibition constants (Fig. 6). The parameters in Fig. 6, A–C, which have an index above 0.75, have the greatest effect on the AUC of itraconazole and its metabolites. Few of the tested parameters were below S10.25, indicating that all have considerable impact on the AUC of itraconazole and its metabolites.

Postanalysis of the Simulated Plasma Profiles

To further quantify the accuracy of the model in predicting the PK profiles for itraconazole and its metabolites, the f1 (deviation observed vs. predicted at identical time points) was calculated. Overall, the PK profiles were predicted adequately, exhibiting f1 of 43%, 30%, and 52% for itraconazole, OH-ITZ, and keto-ITZ, respectively. In Fig. 7 the predicted versus observed concentrations at the same time points are presented.

Model Validation: Prediction of Itraconazole DDI

The results of the simulated clinical trials including midazolam and itraconazole are summarized in Table 2 and Fig. 8. Overall, the model meets the prespecified criteria predicting 100% of observed midazolam AUC and Cmax ratios within 2-fold (Fig. 8A). In addition, 70% of the simulated midazolam AUC ratios were within 1.5-fold...
of the observed data. To further strengthen the DDI validation, other CYP3A4 substrates were also evaluated, confirming the good overall prediction within 2-fold (Fig. 8B; Table 2). The GMFE values were determined to be 1.06 and 0.96 for the AUC and \( C_{\text{max}} \) ratios, respectively. The GMFE values indicate good precision and no bias when predicting the observed DDI.

![Enzyme kinetic plots using human recombinant CYP3A4. Substrate depletion curves vs. time for eight different initial substrate concentrations (different colors). Circles correspond to observed concentrations. The solid lines represent model fitted data from the NLME modeling using the multiple depletions curves method including correction for loss in enzyme activity for itraconazole (A), OH-ITZ (B), keto-ITZ (C), and ND-ITZ (D).](image)

![Percentage remaining activity vs. nominal inhibitor concentration using pooled HLM and midazolam hydroxylation as a CYP3A4 probe reaction: itraconazole (gray), OH-ITZ (black), keto-ITZ (blue), and ND-ITZ (red). The circle and error bars correspond to the observed mean data and S.D. The lines in (A) represent the model fitted data; the lines in (B) represent the simulated free concentrations for itraconazole and its metabolites corrected by protein in the incubation.](image)
Model Application: Relative Contribution to the AUC Ratio

The predicted time course of CYP3A4 inhibition after itraconazole dosing using different formulations was simulated (Supplemental Figs. 3 and 4). The figures illustrate the relationship between simulated unbound liver and gut concentrations and the respective unbound apparent $K_i$ for itraconazole and its metabolites. The corresponding liver average unbound concentration ($C_{\text{unb}}/K_{\text{unb}}$) values (over 96 hours) for itraconazole, OH-ITZ, and keto-ITZ for this dose are 85, 31, and 0.7 for capsule formulation and 200, 62, and 0.85 for oral solution formulation. To gain further insights, the relative contributions of itraconazole and its metabolites to the AUC ratio were calculated and are presented in Fig. 9. In the first hours after dose the main contributor is itraconazole (80%), but as time passes its contribution equals out to a similar level as OH-ITZ (55% vs. 40%). The relative role of OH-ITZ increases with time after itraconazole dosing. The contribution of keto-ITZ is minor throughout the studied 8 day period compared with itraconazole and OH-ITZ (<5%).

Discussion

In the present work, an accurate PBPK model was developed that simultaneously predicts the PK profiles for itraconazole, OH-ITZ, and keto-ITZ. The performance of the model was successfully verified against several PK ($n = 11$) and DDI ($n = 18$) studies, which included different dosing regimens, formulations, and CYP3A4 substrates (Tables 1 and 2). This study is the first to generate in vivo PK data for keto-ITZ as well as in vitro PK data for itraconazole and its three major metabolites, enabling the necessary scaling PK parameters for building a PBPK model. The strengths of this itraconazole PBPK model compared...
The first and most critical step when using PBPK simulations is to accurately predict the PK of the inhibitor and substrate drugs. The model presented meets the prespecified acceptance criteria, with the majority of observed plasma drug concentrations being within the 90% prediction interval (Figs. 4 and 5). Here, the criteria were chosen on the basis of PK variability of itraconazole and its metabolites derived from its complex PK and bioavailability (Poirier and Cheymol, 1998). Being a drug with a broad therapeutic window (Buchkowsky et al., 2005), more flexible criteria are considered acceptable (Jones et al., 2015). There is a lack of good standardization of model acceptance criteria in PBPK modeling (Sager et al., 2015). Herein, one of the methods highlighted in this review has been used for retrospective analysis together with goodness-of-fit plots following best-practice examples (Wagner et al., 2012; Gertz et al., 2013). The calculation of $f_1$ considers all of the observed data points and is a direct comparison of the predicted value at the specific time point (Fig. 7). The $f_1$ calculations confirm the accuracy in PK predictions for itraconazole (43%) and OH-ITZ (30%) and indicate that the prediction of the PK profile of keto-ITZ is less accurate (53%), following the criteria set by Sjögren et al. (2013). As shown in Fig. 7, precision could be improved for the lower concentrations.
hand, high model performance was observed for $C_{\text{max}}$ and steady-state concentrations of itraconazole (31%) and OH-ITZ (21%).

The second step in the present work was to use the validated PK model to assess how well the model could predict AUC and $C_{\text{max}}$ ratios for CYP3A4 substrates with and without the presence of an inhibitor. In general, the predicted versus observed ratios were within 2-fold (Fig. 8B) with good precision and no bias in the DDI prediction showing GMFE values close to 1. There are some specific scenarios where predictions could be further improved, which could also be observed in the Chen et al. (2016) model when the same studies were simulated. For example, when the itraconazole oral dose reached 400 mg an inhibition plateau was observed on day 1 (Templeton et al., 2010). The model overpredicted the AUC ratio and did not capture the plateau (data set 21). To date, the mechanism behind this plateau is not understood and more clinical studies using the 400 mg itraconazole dose would help to clarify this finding. Another example is the general trend to underpredict the DDI in scenarios where the substrate is given more than 12 hours after itraconazole dose (data sets 18, 26, and 27); this could be due to the unrecognized contribution of the last metabolite, ND-ITZ. Nevertheless, in the case of itraconazole and clinical study designs for DDI, the most critical data are the steady-state prediction, where our model showed more accurate performance with 89% of these studies within the more strict criteria of 1.5-fold error (i.e., trials 12 to 13, 15–17, 22 to 23, and 28 to 29 in Table 2).

The role of metabolites in DDI is a developing area of research. The presented in vitro results confirm that all three metabolites are potent inhibitors of CYP3A4 with unbound IC$_{50}$ values in the nanomolar range, comparable to itraconazole IC$_{50}$ (Fig. 3; Table 4). When the ratio between circulating metabolite concentrations and $K_i$ is higher than 0.1, PBPK modeling is recommended (Callegari et al., 2013). All itraconazole metabolites were predicted to have values of the ratio between circulating metabolite concentrations and $K_i$ above 0.1 and to significantly contribute to the observed clinical DDIs by Templeton et al. (2008). Therefore, it can be considered highly relevant to build a PBPK...
model that includes all metabolites, and in this paper we have presented the first step that includes the two first metabolites that are sequentially formed: OH-ITZ and keto-ITZ. The current model enables simulations for hypothesis testing to gain a deeper understanding of the contribution of the metabolites to the observed DDI (Fig. 9). The relative contribution depends on the time window observed after dosing; therefore, this model also enables simulations of the metabolite profiles over time with different trial designs. The relative role of OH-ITZ is increased with time after itraconazole dosing, being similarly important to itraconazole after 12 hours and having even higher contribution after day 3. However, the contribution of keto-ITZ is minor (<5%) over time. Hence, this is the first study by PBPK modeling that assesses in detail the contribution of keto-ITZ and establishes the low impact to the overall inhibition. Nevertheless, the inclusion of this metabolite in the model is indispensable to allow further development including ND-ITZ, which is expected to play a more significant role in the inhibition 12 hours or more following the last dose of itraconazole. Given the long half-life, lower protein binding, and potency of ND-ITZ, it can be predicted that its contribution to the observed DDI is increasing with time (similar to OH-ITZ) and this will be crucial when the CYP3A4 substrate is given more than 12 hours or days after the last dose of itraconazole. Thus, the next step will be to also include the third metabolite (ND-ITZ) to further improve the DDI prediction in those scenarios.
IC50 values that vary between 150- and 170-fold for itraconazole (Heykants et al., 1989; Arredondo et al., 1995, 1999; Ishigam et al., 2001; Templeton et al., 2008). A second example is the reported inhibition of itraconazole, OH-ITZ, and keto-ITZ, but not by other metabolites such as ND-ITZ.

In this report, data are generally presented with good precision on different experimental days (CV % ≤ 30), increasing the confidence in our data. At the same time, the in vitro data are critical when building a mechanistic bottom-up PBPK model (Jamei et al., 2004). As mentioned previously, high-quality in vitro data are required to both evaluate DDI involving new victim compounds and to facilitate optimal study design.

Previous reports (Chen et al., 2016; Liu et al., 2016) have emphasized discrepancies in reported in vitro data. For example, a 30-fold range of IC50 values has been reported for fms in plasma for the parent compound (Heykants et al., 1989; Arredondo et al., 1995, 1999; Ishigam et al., 2001; Templeton et al., 2008). A second example is the reported K_i or IC50 values that vary between 150- and 170-fold for itraconazole and OH-ITZ, respectively (Back and Tjia, 1991; von Moltke et al., 1996; Wang et al., 1999; Ishigam et al., 2001; Tran et al., 2002; Isoherranen et al., 2004). As mentioned previously, high-quality in vitro data are critical when building a mechanistic bottom-up PBPK model (Jamei et al., 2009), and this is emphasized as well by our sensitivity analysis results (Fig. 6). Therefore, key parameters were experimentally determined in the same laboratory at the same occasion for the parent and the metabolites. In this report, data are generally presented with good precision on different experimental days (CV % ≤ 30), increasing the confidence in our data. At the same time, the in vitro data are critical when building a mechanistic bottom-up PBPK model (Jamei et al., 2004). As mentioned previously, high-quality in vitro data are critical when building a mechanistic bottom-up PBPK model (Jamei et al., 2009), and this is emphasized as well by our sensitivity analysis results (Fig. 6). Therefore, key parameters were experimentally determined in the same laboratory at the same occasion for the parent and the metabolites. In this report, data are generally presented with good precision on different experimental days (CV % ≤ 30), increasing the confidence in our data.

The scope for this study was to mechanistically describe the most critical processes for assessing the CYP3A4 inhibition of itraconazole and its metabolites by in vitro-in vivo extrapolation, but it goes without saying that other questions remain to be studied. The contribution of the third metabolite, ND-ITZ, to DDI has been suggested as being clinically relevant (Templeton et al., 2008), but our model does not include it due to limitations of the software. However, in vitro data for ND-ITZ were generated during this study to enable future model development. There are conflicting data on hepatic uptake for itraconazole (Yamano et al., 1999; Higgins et al., 2014). In the current model hepatic uptake was set to 1 following the most recent publication, which showed lack of hepatic uptake in in vitro human primary hepatocytes and knockout mice. However, it could be beneficial to further investigate this to clarify the possibility that carrier-mediated transport might be involved. It is also important to remember that itraconazole and its metabolites are known inhibitors of P-gp and other transporters (Vermeer et al., 2016). The absorption of itraconazole is currently described by first-order kinetics. This limits the simulations on the potential regional differences in the inhibition of intestinal CYP3A4 and transporters. Expansion to a multicompartent gut model is something that could be evaluated and possibly included in future model versions to further improve the mechanistic behavior of the model.

In this paper, a model is presented in which we have successfully included the metabolite keto-ITZ into a PBPK model for itraconazole PK that enables DDI simulations. We believe that this model provides improved mechanistic understanding of the PK and DDI of ITZ and its metabolites. The results presented and sensitivity analyses highlight the importance of robust in vitro and in vivo data to enable complex model building. The predictive DDI risk capability of this model is improved compared with the Simcyp itraconazole library model (100% vs. 80% predicted within 2-fold), showing no bias and good precision. Therefore, our observations suggest that this novel PBPK model built for itraconazole and two of its main metabolites can be successfully used to both evaluate DDI involving new victim compounds and to facilitate optimal study design.

Acknowledgments

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Authorship Contributions

Participated in research design: Prieto Garcia, Kanebratt, Ericsson, Lennernäs, Lundahl.

Conducted experiments: Prieto Garcia.

Performed data analysis: Prieto Garcia, Janzén, Lundahl.

Wrote or contributed to the writing of the manuscript: Prieto Garcia, Janzén, Kanebratt, Ericsson, Lennernäs, Lundahl.

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PBPK of Itraconazole and Metabolites to Predict CYP3A4 DDI

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