Physiologically Based Pharmacokinetic Modeling to Predict Drug-Drug Interactions Involving Inhibitory Metabolite: A Case Study of Amiodarone

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ABSTRACT

Evaluation of drug-drug interaction (DDI) involving circulating inhibitory metabolites of perpetrator drugs has recently drawn more attention from regulatory agencies and pharmaceutical companies. Here, using amiodarone (AMIO) as an example, we demonstrate the use of physiologically based pharmacokinetic (PBPK) modeling to assess how a potential inhibitory metabolite can contribute to clinically significant DDIs. Amiodarone was reported to increase the exposure of simvastatin, dextromethorphan, and warfarin by 1.2- to 2-fold, which was not expected based on its weak inhibition observed in vitro. The major circulating metabolite, mono-desethyl-amiodarone (MDEA), was later identified to have a more potent inhibitory effect. Using a combined “bottom-up” and “top-down” approach, a PBPK model was built to successfully simulate the pharmacokinetic profile of AMIO and MDEA, particularly their accumulation in plasma and liver after a long-term treatment. The clinical AMIO DDIs were predicted using the verified PBPK model with incorporation of cytochrome P450 inhibition from both AMIO and MDEA. The closest prediction was obtained for CYP3A (simvastatin) DDI when the competitive inhibition from both AMIO and MDEA was considered, for CYP2D6 (dextromethorphan) DDI when the competitive inhibition from AMIO and the competitive plus time-dependent inhibition from MDEA were incorporated, and for CYP2C9 (warfarin) DDI when the competitive plus time-dependent inhibition from AMIO and the competitive inhibition from MDEA were considered. The PBPK model with the ability to simulate DDI by considering dynamic change and accumulation of inhibitor (parent and metabolite) concentration in plasma and liver provides advantages in understanding the possible mechanism of clinical DDIs involving inhibitory metabolites.

Introduction

Recently, more attention has been drawn to the perpetrator drug’s metabolites that may also contribute to cytochrome P450 (P450) inhibition (Isoherranen et al., 2009; Yeung et al., 2011; Callegari et al., 2013). In light of the Food and Drug Administration 2012 drug-drug interaction (DDI) draft guidance (http://www.fdagov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362pdf), evaluation of the enzyme inhibition of metabolites which are present at 25% or more of the parent drug area under the curve (AUC) is recommended. Similarly, the European Medicines Agency 2010 DDI guidance (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf) recommends a 20% cutoff. Scientists from 18 IQ (International Consortium Innovation & Quality in Pharmaceutical Development) member companies have formed a working group with one of the goals being to understand the contribution of inhibitory metabolites to P450-mediated DDIs by investigating 140 of the most frequently prescribed drugs. Detailed information for each drug and its reported metabolites, such as the exposure ([I]) and in vitro inhibition potency [Ki (inhibition constant) for reversible inhibitors or k_inact/Ki (maximum inactivation rate constant/inactivation rate constant) for time-dependent inhibitor] for both the parent drug and metabolite(s), and the magnitude of clinical DDIs, was collected through literature searches. Although the majority of DDIs can be traced back to the perpetrator drug itself, eight of 137 drugs were identified as having unexpected clinical DDIs based on the parent drug inhibition potency. Five of these eight drugs—namely, amiodarone, atorvastatin, bupropion, gemfibrozil, and sertraline—show evidence that the underpredicted DDI (based on [I/Ki]) could be due to the omission of the contribution from the inhibitory metabolite(s).

Amiodarone (AMIO) is an effective class III antiarrhythmic agent. AMIO has documented clinical DDIs with comedications, including cyclosporine A, simvastatin, dextromethorphan, metoprolol, and warfarin. It was reported that multiple doses of AMIO (200–400 mg) increased the exposure of simvastatin by 1.7-fold, dextromethorphan by 1.3- to 2-fold, and warfarin by 1.2- to 2-fold (Heimark et al., 1992; Werner et al., 2004; Becquemont et al., 2007). These clinically observed DDIs were not expected based on in vitro competitive inhibition data from AMIO itself (KI > 45 μM for all P450s, using the [I/Ki] method) (Ohyama et al., 2000b). Several in vitro studies, however, indicated the possible inhibitory effect of an AMIO metabolite, mono-desethyl-amiodarone (MDEA) (Ohyama et al., 2000b; McDonald et al., 2012). Considering that MDEA is the major metabolite with plasma exposure in humans comparable to the parent (~1–5 μM at steady state) after long-term treatment (Latini et al., 1984), it was hypothesized that...
inhibition of MDEA may play an important role in amiodarone DDIs with the comedication mentioned earlier.

To gain a mechanistic understanding of how inhibitory metabolites could contribute to the clinically observed AMIO DDIs, a physiologically based pharmacokinetic (PBPK) modeling approach was taken in the present study. The mechanistic PBPK models allow "what-if" scenario analysis that is particularly useful when the in vitro and in vivo experimental data for complex DDIs involving inhibitory metabolite are limited (Sager et al., 2014). Although many clinical pharmacokinetic (PK) studies for AMIO were reported during the 1980s (Kannan et al., 1982; Bonati et al., 1983; Holt et al., 1983), the conventional PK model has not been successful in describing the disposition kinetics of AMIO and its metabolite (Weiss, 1999). Highly variable PK parameters, such as the large volume of distribution (10–65 l/kg) (Latini et al., 1984) and long terminal half-life (16 hours to 58 days after single dose, >50 days following cessation of long-term treatment) (Latini et al., 1984; Marchiset et al., 1985), have been reported. Therefore, one of the main challenges in assessing the DDI caused by AMIO is to be able to model the PK profile of both AMIO and its metabolite, MDEA, with particular emphasis on the accumulation of AMIO and MDEA in plasma and the liver. It has been reported that both AMIO and MDEA have extensive tissue accumulation, especially after long-term therapy, and that the accumulated plasma concentration is several times higher than that predicted from single-dose PK data (Latini et al., 1984). By considering the dynamic change of inhibitor concentration and the accumulation of both parent and metabolite, PBPK modeling may help with understanding the disconnection between in vitro inhibition potency and in vivo clinical DDIs.

The objectives of the present work are 1) to build a PBPK model that can describe the PK profile of AMIO and MDEA, especially their accumulation in plasma and tissue; 2) to simulate the clinically observed DDIs of AMIO with CYP2C9, 2D6, and 3A substrates by considering the inhibition of AMIO alone or including the contribution from the inhibitory metabolite MDEA; and ultimately, 3) to contribute to the understanding of the involvement and impact of inhibitory metabolites for DDIs observed in the clinic.

### Materials and Methods

#### Data Collection for AMIO and MDEA

The in silico and in vitro data used for AMIO and MDEA PBPK model development are shown in Table 1. The reported free fraction of AMIO in plasma is highly variable, ranging from 0.04 to 0.0002 (Laliberté et al., 1984; Veronese et al., 1988). The data generated in house using the RED device (Waters et al., 2008) at 5 μM show that both AMIO and MDEA are highly bound to plasma protein with $f_{u,p} < 0.001$. The free fraction in liver microsome ($f_{u,m}$) measured in house was 0.04 and 0.188, as compared with calculations of 0.4 and 0.58 using the in silico method for AMIO and MDEA, respectively. The model-based sensitivity analysis was conducted to evaluate the effect of variable $f_u$ values on hepatic clearance prediction (see PBPK Model Development section). In vitro metabolic intrinsic clearance ($C_{L, int}$) of AMIO and MDEA was determined in human liver microsome incubations using an in-house assay (Halladay et al., 2007). The $C_{L, int}$ was 28 μl/min per mg for AMIO and 4 μl/min per mg for MDEA incubated at 1 μM of substrate concentration. The enzyme kinetics of AMIO to form MDEA via CYP1A2, 2C8, 2C9, 2D6, and 3A4 were also reported (Ohyama et al., 2000a). Based on reported $K_m$ and $V_{max}$ and using scaling factor and P450 abundance data in the model, the contribution of each P450 to the formation of metabolite MDEA was estimated (Rowland Yeo et al., 2010). The data indicated that CYP3A4 and CYP2C8 are the major enzymes responsible for more than 50% (≥50%) of CYP3A4 and ≥30% of CYP2C8 of the MDEA formation.

In vitro P450 inhibition data reported by Ohyama et al. (2000b) and generated in house are summarized in Table 2. In general, AMIO is a weak reversible inhibitor of CYP2C9, 2D6, and 3A with $K_I$ values of 94.6, 45.1, and 271.6 μM, respectively. However, the metabolite MDEA is a more potent reversible inhibitor with $K_I$ values of 2.3, 4.5, and 12.1 μM, respectively. In addition, MDEA was also reported as a time-dependent inhibitor (TDI) for CYP2D6, and AMIO was reported as a TDI for CYP2C9 and CYP3A4 with $K_I$ and $k_{max}$ values listed in Table 2.

#### PBPK Model Development

A PBPK model was constructed for AMIO and its metabolite MDEA using a population-based absorption, distribution, metabolism, and excretion simulator (V12; Simcyp, Sheffield, UK). To better describe disposition kinetics of AMIO and MDEA, the model was first developed to be able to simulate the

### Table 1: Parameters for AMIO and MDEA used in PBPK modeling

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AMIO</th>
<th>MDEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mol weight (g/mol)</td>
<td>645.32</td>
<td>617.25</td>
</tr>
<tr>
<td>Log $P_{app}$</td>
<td>7.57</td>
<td>7.32</td>
</tr>
<tr>
<td>Compound type</td>
<td>Monoprotic acid</td>
<td>Monoprotic acid</td>
</tr>
<tr>
<td>$pK_a$</td>
<td>6.56</td>
<td>5.58</td>
</tr>
<tr>
<td>$f_{u,p}$</td>
<td>0.73 ± 0.06</td>
<td>0.6</td>
</tr>
<tr>
<td>$f_u$</td>
<td>0.0009</td>
<td>0.0009</td>
</tr>
<tr>
<td>$k_t$ (hour$^{-1}$)</td>
<td>0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>$T_{lag}$ (hour)</td>
<td>1 (Canada and Lesko, 1980; Plomp et al., 1984)</td>
<td>NA</td>
</tr>
<tr>
<td>$k_{p,t}$ of tissues</td>
<td>190/140/220/23/20</td>
<td>75/767/1000/130/32</td>
</tr>
<tr>
<td>$V_{sec}$ (l/kg)</td>
<td>Adipose/liver/lung/heart/kidney, (Plomp et al., 1984); $K_p$ for other organs assumed to be 1</td>
<td>Adipose/liver/lung/heart/kidney, (Plomp et al., 1984); $K_p$ for other organs assumed to be 1</td>
</tr>
<tr>
<td>$f_{u,p}$</td>
<td>0.0009</td>
<td>3.15</td>
</tr>
<tr>
<td>$C_{L, int}$-CYP1A2/2C8/3A4/2C19</td>
<td>1.93/56/13/2.4/79 μl/min per pmol</td>
<td>NA</td>
</tr>
<tr>
<td>$C_{L, int}$</td>
<td>Retrograde calculation in Simcyp to account for 90% of total CL (≈9.4 l/h) toward the formation of MDEA</td>
<td>NA</td>
</tr>
<tr>
<td>$C_{L, int}$</td>
<td>33.5 μl/min per mg</td>
<td>8.25 l/h</td>
</tr>
<tr>
<td>CL$_{iv}$</td>
<td>Negligible</td>
<td>Simcyp best fit</td>
</tr>
<tr>
<td>CL$_{iv}$</td>
<td>(Holt et al., 1983; Plomp et al., 1984)</td>
<td>NA</td>
</tr>
</tbody>
</table>

$P_{app}$, blood-to-plasma ratio; $CL_{int}$, clearance; $CL_{iv}$, renal clearance; $f_{u,p}$, unbound fraction in human plasma protein; $f_{u,m}$, fraction of drug unbound in the gut; $f_{u,p}$, free fraction in plasma; HSA, human serum albumin; $k_t$, first-order absorption rate constant; NA, not available; $T_{lag}$, lag time; $V_{sec}$, volume of distribution of compartment.
PK of AMIO and MDEA formation after intravenous administration of AMIO, from which best estimates of clearance (CL) and volume of distribution at steady state (Vss) were obtained. Then the model was expanded to include absorption to simulate the oral PK of AMIO and formation of MDEA after single and multiple doses. The verified PK model was then used for DDI prediction by considering the inhibitory effect of both AMIO and its metabolite MDEA.

Even though many clinical PK studies for AMIO were reported in the 1980s, very limited full concentration-time profile data, especially for metabolite MDEA, were available. Clinical PK data from various studies, even with very small sample sizes, were used in the model development (Canada and Lesko, 1980; Holt et al., 1983; Latini et al., 1984; Plomp et al., 1984) and verification (Andreasen et al., 1981; Staubli et al., 1983; McDonald et al., 2012). Detailed model input parameters with source information for clinical study are also listed in Table 1.

**Model for AMIO and MDEA PK after Intravenous Administration.**

A full PBPK distribution model was built for AMIO to best describe the multieponential PK profile with long terminal half-life. The Vss of AMIO was predicted using a mechanistic tissue composition equation (Rodgers and Rowland, 2006) built in Simcyp, with input tissue plasma partition coefficient (k_p) estimated based on tissue distribution of AMIO in adipose, liver, lung, heart, and kidney observed in human subjects (Plomp et al., 1984). k_p values of all other organs for which no clinical accumulation data were reported are set as 1.

AMIO is cleared primarily through hepatic metabolism, less via biliary excretion, and negligibly in urine (Holt et al., 1983; Latini et al., 1984). The reported in vivo CL is variable, mostly ranging from 8 to 46 l/h, depending on the length of plasma sampling in the clinical study and PK model used (Riva et al., 1982; Latini et al., 1984). A higher value of CL was reported mostly from studies with a short sampling period and/or insufficient terminal data points used in PK modeling. The metabolic CL predicted from in vitro data using in vitro–in vivo extrapolation (IVIVE) with the well stirred model ranges 2.6–9.3 l/h, depending on the plasma protein and microsomal binding data used, 0.0009–0.04 for free fraction (f_u) and 0.04–0.4 for f_u,mic, respectively. These values are on the lower end of the observed range. The CL of 9.3 l/h obtained from IVIVE, which is close to the lower end of reported values, was used in the initial model development. The final estimate of CL was determined to be the value that allows the model to best recover the observed PK profile in the simulations using predicted Vss. Since AMIO is primarily eliminated through metabolic pathways, and MDEA is the predominant metabolite, the model assumed ∼90% of AMIO metabolic CL going to the formation of metabolite MDEA, mainly by CYP3A4 (∼50%), followed by CYP2C8 (∼30%), and CYP1A2 and CYP2C19 (∼10%). The remaining ∼10% of AMIO metabolic CL was assigned to additional metabolism. The model inputs for each P450 as CL_{int} back calculated using a Simcyp retrograde calculator are listed in Table 1.

The metabolite MDEA was modeled using a minimal-PBPK distribution model (Cao and Jusko, 2012). To more accurately describe the shape of the concentration-time profile of MDEA, the minimal-PBPK distribution model with the addition of a single adjusting compartment set in Simcyp was adapted. The Vss was predicted by inputting the k_p of MDEA in adipose, kidney, liver, lung, and heart estimated from clinical data (Plomp et al., 1984) into the mechanistic tissue composition equation (Rodgers and Rowland, 2006) built in Simcyp. The volume of distribution of compartment (V_{int}) was estimated and optimized based on best fitting of the observed time-concentration profile of MDEA.

The formation of MDEA was driven by the metabolic clearance of AMIO described earlier. The CL of MDEA determined using IVIVE from in vitro human liver microsome data is ∼1 l/h, much lower than the rate of formation clearance from AMIO (∼8 l/h) reported. Considering that the observed half-life for MDEA is similar to or slightly longer than that for AMIO (Holt et al., 1983; Latini et al., 1984), it can be assumed that the kinetics of MDEA are more formation rate–limited. Therefore, the value of CL at −8 l/h (close to the formation CL from AMIO) was used as the initial input in the MDEA model development. The final value for this unknown parameter was determined based on the simulation that can best recover the observed PK profile of MDEA (Table 1).

**Model for AMIO and MDEA PK after Single and Multiple Oral Doses.**

The AMIO-MDEA–linked PBPK model that can well capture the intravenous dose PK of AMIO and MDEA formation was then used to simulate PK of AMIO and MDEA after single and multiple oral doses (Jamei et al., 2009). The reported fraction and rate of absorption (f_a and k_a; Table 1) were used because the permeability data are not available for prediction due to low recovery in the Madin-Darby canine kidney assay conducted in house. The CL and Vss remained the same as determined based on the intravenous PK simulation. The ability of the model to recover the PK of AMIO and MDEA after oral dosing was verified by comparing the simulated data with the observed data.

**Simulation of PK and DDI**

The simulation was conducted using the developed model implemented in Simcyp (V12). The Sim-Healthy volunteer population with randomly selected individuals aged 20–50 years with a gender ratio of 1:1 was used for the PK and DDI simulation. The trial size used in the simulation was according to each clinical study detailed in the following sections.

**Simulation of PK.** For the initial PK model development, a total of 10 trials of 6 subjects receiving a single intravenous infusion (10 minutes) of 400 mg of AMIO were simulated. The simulated PK profiles for AMIO and MDEA were compared with observed data from Holt et al. (1983). The model was then applied to simulate the PK of AMIO and MDEA formation following a single oral dose of 400 mg of AMIO with a trial size of 10 (trial) × 7 (subject) and 14 weeks' oral dosing of 350 mg of AMIO with a trial size of 10 × 3, respectively. Ten separate trials were generated in simulation to evaluate variability across different trial groups. The predictability of the model for oral dosing PK was verified by comparing the predicted PK profile with the observed data from Andreasen et al. (1981) and McDonald et al. (2012).

**Simulation of DDI.** The DDI simulations were conducted using the dose regimen described in the clinical DDI study reports (O’Reilly et al., 1987; Heimark et al., 1992; Werner et al., 2004; Becquemont et al., 2007). In brief, the interactions between AMIO (300 mg, every day (QD) for 13 days or 200 mg twice a day (BID) for 14 days) and warfarin (0.75 mg/kg, day 4), AMIO (1200 mg, QD for 6 days) and metoprolol (119 mg, QD, day 6), and AMIO (400 mg, QD for 4 days) and simvastatin (40 mg, day 4) were simulated with 10 trials each containing 5, 10, and 12 subjects, respectively. The following scenarios were simulated as listed in Table 3: 1) competitive inhibition by AMIO only, 2) competitive inhibition

**TABLE 2**

<table>
<thead>
<tr>
<th>Inhibition of CYP2C9</th>
<th>Inhibition of CYP2D6</th>
<th>Inhibition of CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_i (µM)</td>
<td>TDI (K_i k_{int})</td>
<td>K_i (µM)</td>
</tr>
<tr>
<td>AMIO 94.6</td>
<td>7.89 µM, 0.005 min^{-1}</td>
<td>45.1</td>
</tr>
<tr>
<td>MDEA 2.3</td>
<td>No</td>
<td>45.1</td>
</tr>
</tbody>
</table>

*In-house data following the methodology referred to Sodhi et al. (2014); all other data from Ohyama et al. (2000b). K_i and K_p here are apparent values, and are corrected for free fraction in microsome (f_u,mic = 0.012) estimated in the Simcyp model.
by AMIO and MDEA, 3) both competitive and time-dependent inhibition by AMIO only (for CYP2C9 and CYP3A), and 4) competitive and time-dependent inhibition from both AMIO and MDEA (CYP2C9 and CYP2D6). The simulated AUC and C\text{max} ratio were compared with the observed data.

**Results**

**Simulation of AMIO and MDEA PK after Single Intravenous Dose**

The PBPK model built for AMIO was able to simulate the PK profile observed from clinical study following a 400-mg i.v. infusion of AMIO (Fig. 1A). The very large volume of distribution predicted (V\text{ss} = 64 l/kg) using the full PBPK distribution model by considering extensive tissue/plasma partitioning in major organs reasonably captured the distribution phase that drives the PK profile, with a multiexponential decline and long terminal half-life (t\text{1/2}). The predicted V\text{ss} (64 l/kg) is on the high end of reported values mostly ranging from 12 to 65 l/kg (Riva et al., 1982; Holt et al., 1983). The CL for AMIO is ~9.4 l/h, estimated from simulation that best recovers the observed PK data, and is close to the lower end of the range of reported values (8–46 l/h). This rate of AMIO elimination determines the rate of MDEA formation.

The PBPK model for the metabolite MDEA was developed using in silico, in vitro, and in vivo data (Table 1). MDEA was predicted to also have a large volume of distribution (V\text{ss} = 47 l/kg) based on its extensive tissue distribution data that were reported. The estimate of MDEA CL from the simulation with best fit was ~8.3 l/h. This value is close to that for AMIO (9.4 l/h), which is consistent with the assumption that the kinetic for MDEA PK is mostly formation rate–limited based on the observed similar t\text{1/2} for AMIO and MDEA. The optimized minimal-distribution model with the addition of a single adjusting compartment (volume of distribution of compartment [V\text{ss}c] = ~31 l/kg) captured the shape of the MDEA concentration-time curve that exhibited a multiexponential decline with a long terminal half-life (Fig. 1A).

At a 400-mg intravenous infusion of AMIO, the formation of MDEA, expressed as C\text{max} and AUC, was simulated to be 0.08 µg/ml (0.13 µM) and 37.6 hours*µg/ml (60.9 µM*h), compared with the observed values of 0.09 µg/ml (0.16 µM) and 39.8 hours*µg/ml (64.5 µM*h), respectively (Holt et al., 1983). The time for MDEA to reach its maximum concentration as determined by the rate of AMIO elimination and MDEA clearance is about 42 hours (mean), which is in the range of observed values (24–72 hours) (Holt et al., 1983).

**Simulation of AMIO and MDEA PK after Single and Multiple Oral Doses**

The AMIO-MDEA–linked PBPK model with CL and V\text{ss} for both AMIO and MDEA characterized based on the intravenous PK profile was then used to simulate the PK profile of AMIO and MDEA after oral administration of AMIO. The parameters of fa (0.6), k\text{a} (0.3 hour\textsuperscript{-1}), and time lag (1 hour) reported from clinic studies were incorporated into the model (Canada and Lesko, 1980; Plomp et al., 1984). The simulated AMIO PK profile and parameters of t\text{max} (7.25 hours), C\text{max} (0.43 µg/ml) (0.66 µM), and AUC (6.6 hours*µg/ml) (10.2 µM*h) are in good agreement with the mean observed data [7.3, 0.46 (0.71 µM), and 7.9 (12.2 µM*h), respectively] from a single 400-mg oral dose of AMIO (Andreasen et al., 1981; Latini et al., 1984) (Fig. 2A). The predicted formation of MDEA does not completely reach maximum concentration for over 24 hours (Fig. 2B), similar to the clinical observation (Staubli et al., 1985; McDonald et al., 2012). However, no concentration-time profile data for MDEA from this same study are available for direct comparison.

The PK profile of AMIO and MDEA after long-term treatment was also simulated and compared with reported data (McDonald et al., 2012). The accumulation of AMIO and, in particular, the constantly increasing MDEA level over long-term treatment were captured by the PBPK model simulation. The simulated mean plasma concentration of AMIO and MDEA following a dose of 350 mg/day AMIO for 14 weeks was comparable to the observed mean plasma concentration (n = 3) in samples collected once every 3 weeks during 14 weeks of treatment (Fig. 3A and B). The AMIO dose in the clinical study varied between 230 and 500 mg/day over the treatment period. An overall mean value of 350 mg/day was used in the simulation.

**Simulation of DDI**

The PBPK model with incorporation of in vitro inhibition potency data (Table 2) from AMIO and MDEA on CYP2C9, 2D6, and 3A was used to simulate the effect of AMIO on substrate warfarin (CYP2C9),

### TABLE 3

Comparison of predicted and observed DDI data (AUC and C\text{max} ratio)

<table>
<thead>
<tr>
<th></th>
<th>AUC Ratio</th>
<th>C\text{max} Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin +/− AMIO (400 mg, QD)</td>
<td>Predicted</td>
<td>No interaction</td>
</tr>
<tr>
<td>AMIO comp. inh.</td>
<td>1.27</td>
<td>1.02 (1.01–1.02)</td>
</tr>
<tr>
<td>AMIO comp. inh. + MDEA comp. inh.</td>
<td>2.11</td>
<td>N/A</td>
</tr>
<tr>
<td>Observed (O’Reilly et al., 1987; Heimark et al., 1992)</td>
<td>1.18</td>
<td>1.02 (1.01–1.02)</td>
</tr>
<tr>
<td>Metoprolol +/− AMIO (1200 mg, QD)</td>
<td>Predicted</td>
<td>No interaction</td>
</tr>
<tr>
<td>AMIO comp. inh.</td>
<td>1.73</td>
<td>1.93 (1.8−2.0)</td>
</tr>
<tr>
<td>AMIO comp. inh. + MDEA comp. inh.</td>
<td>2.01</td>
<td>N/A</td>
</tr>
<tr>
<td>Observed (Becquemont et al., 2007)</td>
<td>1.93</td>
<td>2.01</td>
</tr>
<tr>
<td>Metoprolol +/− AMIO (1200 mg, QD)</td>
<td>Predicted</td>
<td>No interaction</td>
</tr>
<tr>
<td>AMIO comp. inh.</td>
<td>1.59</td>
<td>2.66 (1.46–1.59)</td>
</tr>
<tr>
<td>AMIO comp. inh. + MDEA comp. inh.</td>
<td>2.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Observed (Werner et al., 2004)</td>
<td>2.01</td>
<td>2.0</td>
</tr>
</tbody>
</table>

AUC ratio, AUC in the presence of inhibitor/AUC in the absence of inhibitor; AUC\text{0−24}, area under the curve from zero to 24 hours; AUC\text{0−last}, area under the curve from zero to the last time point; C\text{max} ratio, C\text{max} in the presence of inhibitor/C\text{max} in the absence of inhibitor; comp. inh., competitive inhibition; N/A, not available.
metoprolol (CYP2D6), and simvastatin (CYP3A4). Different doses were used for each simulation to match the reported clinical DDI studies. The simulated effect, expressed as a ratio of AUC and $C_{\text{max}}$ in the presence and absence of AMIO, was compared with observed data and presented in Table 3. In addition, the simulation of DDIs by considering all plausible mechanisms of P450 inhibition by AMIO and MDEA can be found in the Supplemental Material.

**Effect of AMIO on Warfarin PK.** The effect of AMIO on the PK of warfarin after a 300-mg QD dose for 13 days or 200-mg BID dose for 14 days was simulated. As expected, AMIO was predicted to have no effect on warfarin PK if only competitive inhibition by AMIO ($K_i$ 96 $\mu$M) was considered. With incorporation of competitive inhibition from both AMIO and MDEA ($K_i$ 2.3 $\mu$M), and the time-dependent CYP2C9 inhibition by AMIO ($k_{\text{inact}}$ 0.005 minute$^{-1}$, $K_I$ 7.89 $\mu$M), the model predicted a mean of 14 and 18% increase in the AUC of warfarin compared to the observed 27 and 100% increase after 300-mg QD and 200-mg BID doses, respectively. Even though the predictions were improved after including the inhibitory metabolite, and the AUC ratio predicted is within 2-fold of the observed value, a trend of underprediction for 200 mg BID was observed (Table 3).

**Effect of AMIO on Metoprolol PK.** The effect of AMIO on the PK of metoprolol after a 1200-mg QD dose for 6 days was simulated. When CYP2D6 inhibition from metabolite MDEA was not counted, AMIO alone ($K_i$ 45.1 $\mu$M) was predicted to have no effect on the metoprolol PK. Even with incorporation of competitive inhibition for both AMIO and MDEA, a minimal effect of AMIO on metoprolol was predicted (1.03 and 1.02 in AUC and $C_{\text{max}}$ ratios, respectively), whereas when both competitive and time-dependent inhibition ($k_{\text{inact}}$ 0.12 minute$^{-1}$, $K_I$ 1.3 $\mu$M) from MDEA were incorporated, significant DDI was predicted with the $C_{\text{max}}$ ratio (1.53) and AUC ratio (2.45), slightly higher than the clinical observed data (Table 3).

**Effect of AMIO on Simvastatin PK.** The interaction between AMIO and simvastatin following a 400-mg QD dose of AMIO for 4 days was simulated using the model with the updated value of gut availability (0.4) for simvastatin. The model predicted no effect of AMIO on simvastatin PK when only the competitive inhibition of AMIO was incorporated. The model predicted a 1.93-fold increase in both simvastatin $C_{\text{max}}$ and AUC when competitive inhibition from both AMIO and MDEA is considered. These values are similar to the observed data: 2.0- and 1.73-fold increase in $C_{\text{max}}$ and AUC, respectively (Table 3). Interestingly, if both CYP3A4 competitive and time-dependent inhibition from AMIO and reversible inhibition from MDEA were incorporated, the model predicted a more than 2-fold inhibition for both $C_{\text{max}}$ (3.0-fold) and AUC (3.5-fold) of simvastatin.

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**Fig. 1.** Simulated and observed plasma concentration-time profiles of AMIO (A) and MDEA (B) following a single 400-mg i.v. administration. The black line represents the mean concentration for the simulated population (10 trials × 6 subjects). The thin gray lines represent simulated individual trials. The triangles denote the mean values ($n = 6$) from the clinical trials (Holt et al., 1983).

**Fig. 2.** Simulated and observed plasma concentration-time profiles of AMIO (A) and MDEA (B) following a single oral dose of 400 mg of AMIO. The black line represents the mean concentration for the simulated population (10 trials × 7 subjects). The thin gray lines represent simulated individual trials. The triangles denote the mean values ($n = 7$) from the clinical trials (Andreasen et al., 1981).
Discussion

Even though the underestimation of clinical DDI solely due to the overlooking of contributions from inhibitory metabolites is uncommon, when unexpected DDI is observed in clinical studies, the potential contribution of metabolites to the observed DDI should be evaluated. Obtaining a mechanistic understanding of inhibitory metabolite contributions to the observed DDI is important, and the use of PBPK modeling could be advantageous, especially considering that complex drug interactions involving inhibitory metabolite as the model will allow the considerations of nonlinear PK, accumulation of inhibitor concentration (parent and metabolites) over the treatment period, and extensive tissue distribution. However, the use of the PBPK model for a true prospective prediction of DDI caused by inhibitory metabolite is still very challenging, as the in vitro inhibition and human PK data for the metabolite are not routinely generated. The AMIO case study presented here demonstrates the utility of PBPK modeling in a retrospective way to gain a mechanistic understanding of the unexpected clinical DDI potentially caused by an inhibitory metabolite. The development of the PBPK model from additional case studies will prove useful for the prediction of DDI involving inhibitory metabolite in the future.

The disposition kinetics of AMIO were poorly understood until sensitive bioanalytical methods were developed, long after the drug was approved. AMIO and its metabolite, MDEA, have unique pharmacokinetic properties characterized by extensive tissue distribution, very long half-life (up to 100 days), and accumulation during chronic oral therapy. In addition, nearly 100-fold variation in the reported PK parameters, such as terminal half-life and steady-state volume of distribution, may also be due to inappropriate PK modeling (McDonald et al., 2012). Therefore, characterizing the concentration-time profile of inhibitors, AMIO and its metabolite MDEA, in plasma and liver after single and multiple doses is the first and critical step toward understanding the underestimated clinical DDI using a PBPK modeling approach.

The PBPK model developed using a mixed “bottom-up” and “top-down” approach leverages the use of all existing AMIO and MDEA in vitro absorption, distribution, metabolism, and excretion data and clinical PK data. Large Vss values were predicted for both AMIO and MDEA by incorporating tissue distribution into mechanistic tissue composition equations (Rodgers and Rowland, 2006). The use of a full PBPK distribution model for AMIO and minimal PBPK + single adjusting compartment distribution model for MDEA enabled a good description of the multiphasic decline of the concentration-time profile and long terminal t1/2 for both AMIO and MDEA following an intravenous infusion of AMIO. The AMIO-MDEA–linked PBPK model predicted both the formation and elimination of metabolite MDEA successfully, as the observed and simulated MDEA PK profiles match reasonably well. More importantly, the accumulation of AMIO and MDEA in plasma and liver after chronic oral dosing was captured in the simulations as well. The simulated mean plasma concentration increased ~3-fold for AMIO and ~20-fold for MDEA after 8 weeks of a QD dose of AMIO (Fig. 3). At steady state, the simulated plasma concentration of metabolite MDEA reached a similar level as the parent AMIO (~1 μg/ml), which is very similar to the clinical observations (Holt et al., 1983; McDonald et al., 2012). Moreover, the simulated total liver concentrations for both AMIO and MDEA are several hundred-fold higher than that in plasma due to extensive tissue distribution considered in the model (Fig. 4, A and B). The accumulation of MDEA in the liver is more than that of AMIO due to higher liver-plasma partitioning of MDEA incorporated in the model based on reported data (Holt et al., 1983). Overall, the dynamic change of inhibitor (AMIO and MDEA) concentration over time and its accumulation in plasma and liver, especially for inhibitory metabolite MDEA, were characterized in the PBPK model. The successful simulations of clinically observed PK profile build confidence in the prediction and mechanistic understanding of the DDI caused by AMIO, particularly the DDI potential in chronic therapy.

In general, the addition of the inhibitory metabolite, MDEA, into the PBPK model resulted in more accurate prediction of all AMIO DDIs (AUC and Cmax ratio) than that only considering parent drug (AMIO) P450 inhibition. However, considering that the magnitude of DDIs observed in clinic is rather small (mostly 1.2- to ~2.0-fold) and the PK parameters reported for AMIO are largely variable, more clinical study data would be useful to build up confidence in the model prediction. Nevertheless, for the AMIO-metoprolol DDI, when the competitive inhibition of AMIO and competitive and time-dependent inhibition of MDEA were considered, the model provided the best prediction. Nearly no effect of AMIO on metoprolol PK would be predicted if TDI of MDEA was not incorporated, which suggests that the underlying mechanism for the AMIO-metoprolol interaction may be more attributable to the TDI by MDEA on CYP2D6.
CYP2D6 and CYP3A DDIs were well predicted suggests that underprediction of CYP2C9 DDI is more likely due to other unknown mechanisms or the variability of clinical DDI data from a small trial ($n = 5$). Recently, several other metabolites have been identified in plasma samples of subjects receiving AMIO, including $3'$-hydroxy-$N$-monodesethylamiodarone, $N,N$-didesethylamiodarone, and deaminated amiodarone, $O$-desalkylamiodarone. These metabolites have equal (deaminated amiodarone, $3'$-hydroxy-$N$-monodesethylamiodarone) or much more potent ($N,N$-didesethylamiodarone, $O$-desalkylamiodarone) CYP2C9 inhibition than MDEA (McDonald et al., 2012). The contribution of these metabolites to the observed in vivo AMIO-warfarin interaction is yet to be assessed.

The current study investigated the impact of adding inhibitory metabolite on the magnitude of the predicted DDIs while all rest parameters were kept consistent as either defined in the verified PK model or default Simcyp model. Although this retrospective PBPK modeling helped to understand the possible inhibition mechanism of clinically observed AMIO DDI, the prospective prediction of DDI involving inhibitory metabolite would require further development of the PBPK model. Although it is reasonable to assume that the free liver concentration could be similar to the free plasma concentration when a perfusion-limited model is used, the use of this assumption to determine the metabolite concentration at interacting sites is still uncertain, especially for a highly lipophilic metabolite such as MDEA. Other parameters, such as free inhibitory metabolite concentration in the gut (Yang et al., 2007; Karlsson et al., 2013) and in vivo inhibition potency of the inhibitory metabolite, can also affect the prediction. A good understanding of the source of uncertainty for these parameters and its impact on the prediction is important. In addition, understanding and incorporating the metabolic clearance pathways of the inhibitory metabolite could be critical if the autoinhibition by the inhibitory metabolite exists.

This work demonstrates the utility of PBPK modeling in developing mechanistic understanding of the clinical DDI attribute to the contribution of inhibitory metabolite. Accurate simulation of PK profiles of both parent and metabolite is required to maximize confidence in the DDI prediction. Refinement of the PK model, especially for the metabolite, should be conducted whenever more in vitro enzyme kinetic and inhibition data and clinical PK data become available. In discovery and early development stages, prospective prediction of DDI involving an inhibitory metabolite is challenging and has inherent uncertainty due to limited information available for the metabolite, and therefore, hypothesis-driven sensitivity analysis is highly recommended to understand the extent of DDI risk. It also emphasizes the need for users to be fully aware of the model assumptions and limitations as well as underlines the importance of comparing PBPK model predictions with those from simpler models to understand the differences in outcome. In a late stage of development, with full validation of the PK model, PBPK modeling can provide greater value to address clinical questions, such as why more significant DDI was observed than was expected, through simulating the proposed mechanism of inhibition from both parent and metabolites to understand the disconnect.

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