Quantitative Rationalization of Gemfibrozil Drug Interactions: Consideration of Transporters-Enzyme Interplay and the Role of Circulating Metabolite Gemfibrozil 1-O-\(\beta\)-Glucuronide

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ABSTRACT

Gemfibrozil has been suggested as a sensitive cytochrome P450 2C8 (CYP2C8) inhibitor for clinical investigation by the U.S. Food and Drug Administration and the European Medicines Agency. However, gemfibrozil drug-drug interactions (DDIs) are complex; its major circulating metabolite, gemfibrozil 1-O-\(\beta\)-glucuronide (Gem-Glu), exhibits time-dependent inhibition of CYP2C8, and both parent and metabolite also behave as moderate inhibitors of organic anion transporting polypeptide 1B1 (OATP1B1) in vitro. Additionally, parent and metabolite also inhibit renal transport mediated by OAT3. Here, in vitro inhibition data for gemfibrozil and Gem-Glu were used to access their impact on the pharmacokinetics of several victim drugs (including rosiglitazone, pioglitazone, cerivastatin, and repaglinide) by employing both static mechanistic and dynamic physiologically based pharmacokinetic (PBPK) models.

The 48 cases evaluated using the static models, about 75% and 98% of the DDIs were predicted within 1.5- and 2-fold of the observed values, respectively, when incorporating the interaction potential of both gemfibrozil and its 1-O-\(\beta\)-glucuronide. Moreover, the PBPK model was able to recover the plasma profiles of rosiglitazone, pioglitazone, cerivastatin, and repaglinide under control and gemfibrozil treatment conditions. Analyses suggest that Gem-Glu is the major contributor to the DDIs, and its exposure needed to bring about complete inactivation of CYP2C8 is only a fraction of that achieved in the clinic after a therapeutic gemfibrozil dose. Overall, the complex interactions of gemfibrozil can be quantitatively rationalized, and the learnings from this analysis can be applied in support of future predictions of gemfibrozil DDIs.

The current European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) guidelines on DDIs recommend in vitro investigation of metabolite’s interaction potential if present at ≥25% of the parent area under the plasma concentration-time curve (AUC) to reduce the risk for unexpected clinical DDI as a result of not assessing in vitro cytochrome P450 (P450) inhibition by metabolite(s) is low, which is consistent with earlier reports.

ABBREVIATIONS:

ADME, absorption, distribution, metabolism, and excretion; AUC, area under the plasma concentration-time curve; AUCR, area under the plasma concentration-time curve ratio; CLint,bil, biliary intrinsic clearance; CLint,int, intrinsic metabolic clearance; CLint, hepatic clearance; CLint, renal clearance; DDI, drug-drug interactions; Fabs, fraction absorbed; Fdose, fraction of drug escaping gut-wall extraction; Fes, fraction of drug escaping hepatic extraction; fu,gut, fraction unbound in the gut; Gem-Glu, gemfibrozil 1-O-\(\beta\)-glucuronide; fr, maximum unbound plasma perpetrator concentration; fmax, maximum unbound perpetrator concentration at the inlet to liver; kdeg, disappearance rate constant; kdeg,h, apparent first-order degradation rate constant; ki, inhibition constant; km, maximal inactivation rate constant; kib, inhibitor concentration that supports half the maximal rate of inactivation; L, organic anion transporter; OATP, organic anion transporting polypeptide; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic; PSactive, active uptake clearance; PSpass, passive diffusion; Qgut, enterocyte blood flow; Rb, blood-to-plasma ratio; SFactive, scaling factor for active uptake; TDI, time-dependent inhibition; Vss, volume at steady-state.
to focus on the development of mechanistic models for the perpetrator drug/metabolite pairs for amiodarone (Chen et al., 2015), bupropion, gemfibrozil, and sertraline.

Gemfibrozil, when administered at a total daily dose of 900 or 1200 mg, improves lipid and apolipoprotein profiles, particularly very-low-density lipoprotein triglyceride and high-density lipoprotein cholesterol levels, in all types of dyslipidemia (except type I) (Spencer and Barradell, 1996). Gemfibrozil is thus a highly prescribed and well-tolerated drug; however, its concomitant use with repaglinide is contraindicated because of documented drug interactions (Niemii et al., 2003b). As a perpetrator drug, gemfibrozil presents a complex profile involving inhibition of multiple transporters and cytochrome P450 2C8 (CYP2C8) by parent and a circulating metabolite, gemfibrozil 1-O-β-glucuronide (Gem-Glu). Cerivastatin was withdrawn from the worldwide market in 2001 because of a higher incidence of fatal rhabdomyolysis linked to increased exposure caused by gemfibrozil (Farmer, 2001). Reports suggested that 12 of the 31 patients who died had also been taking gemfibrozil along with cerivastatin.

Subsequently, several studies were conducted to understand the mechanisms involved, and it was determined that gemfibrozil (twice a day for 5 days) increases cerivastatin oral AUC about 5-fold, which however was not assumed from the interaction potency of gemfibrozil alone (Backman et al., 2002). Similar DDIs have been observed with repaglinide (~7-fold increase in AUC) (Niemii et al., 2003b; Honkalammii et al., 2011a), pioglitazone (~4-fold increase in AUC) (Aquilante et al., 2013), and rosiglitazone (~2.5-fold increase in AUC) (Niemii et al., 2003a). The results of complimentary in vitro mechanistic studies suggest that gemfibrozil dosing leads to CYP2C8 inhibition, which is attributed to time-dependent inhibition (TDI) by its major circulating metabolite, Gem-Glu (Ogilvie et al., 2006). Furthermore, both parent and metabolite have also been shown to behave as moderate inhibitors of organic anion transporting polypeptide 1B1 (OATP1B1), renal organic anion transporter 3 (OAT3), and weakly inhibit cytochrome P450 2C9 (CYP2C9) and cytochrome P450 3A4 (CYP3A4) (Fujino et al., 2003; Shitara et al., 2004). Consequently, quantitative rationalization of gemfibrozil DDIs is challenging, and requires extensive in vitro characterization and mechanistic integration of all associated interaction components.

Rich clinical data are available, with several DDI studies reported for gemfibrozil, which is now suggested as the clinical probe inhibitor for CYP2C8 activity by the FDA, the EMA, and other agencies (CDER, 2012; CHMP, 2012). Given the complexity of gemfibrozil DDIs, the main objectives of the present study are 1) to mechanistically rationalize the clinical DDIs with a goal to apply the quantitative understanding in the prospective prediction of gemfibrozil interactions before human dosing and enable subsequent clinical study planning and 2) assess the contribution of the circulating metabolite to the magnitude of DDIs. By leveraging information from 48 different clinical DDI studies reported for gemfibrozil, in conjunction with the available in vitro interaction data for parent and metabolite, we could employ both static mechanistic and dynamic physiologically based pharmacokinetic (PBPK) modeling approaches to predict the impact of gemfibrozil on the oral pharmacokinetics of several victim drugs, including rosiglitazone, pioglitazone, cerivastatin, and repaglinide.

Materials and Methods

Clinical DDI and In Vitro Data Collection

Clinical DDI data with gemfibrozil as perpetrator drug were primarily extracted from the University of Washington metabolism and transporter drug interaction database (www.druginteractioninfo.org). An additional exhaustive literature search was conducted to enrich the clinical DDI dataset. In vitro interaction potency data were collected from the scientific literature.

Static Model Predictions

The area under the plasma concentration-time curve ratio (AUCR) of an oral victim drug, in the presence (AUCpo) and absence (AUCpo) of perpetrator, can be described by the following equations:

\[ AUCR = \frac{Fa'}{Fa} \times \frac{Fg'}{Fg} \times \frac{Fh'}{Fh} \times \left( \frac{CLh + CLr}{CLr} \right) \]  

(1)

where

\[ Fh = 1 - \frac{CLh}{Qh} \]

\[ CLh = \frac{Qh}{f_{db,h}CL_{int,h}} \]

\[ CLr = f_{db,GFR} + CL_{sec} \]

\[ Fc, \ Fg, \ and \ Fh \ represent \ the \ fraction \ of \ drug \ absorbed, \ fraction \ of \ drug \ escaping \ gut-wall \ extraction, \ and \ hepatic \ extraction, \ respectively. \ Fc', \ Fg', \ and \ Fh' \ are \ the \ corresponding \ parameters \ in \ the \ presence \ of \ the \ perpetrator, \ CL_{int,h} \ is \ the \ intrinsic \ hepatic \ clearance, \ f_{db,h} \ is \ the \ fraction \ unbound \ in \ blood, \ and \ Qh \ is \ the \ hepatic \ blood \ flow \ [20.7 \ ml/min/kg \ (Kato \ et \ al., \ 2003)]. \ Assuming \ no \ or \ negligible \ tubular \ reabsorption, \ renal \ clearance \ is \ expressed \ as \ a \ function \ of \ the \ glomerular \ filtration \ rate \ (GFR, \ 1.78 \ ml/min/kg) \ and \ active \ secretion \ (CL_{sec}). \]

Net-Effect Model. Gemfibrozil interactions occur primarily at the level of the liver (OATP1B1 and CYP2C8 inhibition) and kidney (OAT3 inhibition). For victim drugs not subjected to active hepatic uptake, the AUCR was predicted using the static net-effect model:

\[ AUCR = \frac{Fa'}{Fa} \times \frac{Fg'}{Fg} \times \left( \frac{CL_{int,h} + CL_{sec}}{CL_{sec}} \right) \times \left( \frac{1}{f_{hepatic} + f_{renal}} \right) \times \left( 1 - \frac{\sum fm_{CYP}}{1 - \sum fm_{CYP}} \right) \]  

(2)

where

\[ f_{hepatic} + f_{renal} = 1 \]

\[ \sum fm_{CYP} + (1 - \sum fm_{CYP}) = 1 \]

and

\[ f_{sec} + f_{GFR} = 1 \]

The terms \( f_{hepatic} \) and \( f_{renal} \) are the fraction of parent drug cleared by hepatic metabolism and renal clearance after oral dosing in the absence of inhibitor drug, respectively. The term \( fm_{CYP} \) is the fraction of the \( f_{hepatic} \) metabolized by a particular CYP enzyme. Assuming no tubular reabsorption, \( f_{sec} \) and \( f_{GFR} \) are the term \( fm_{CYP} \) fraction of parent drug cleared by active secretion and glomerular filtration, respectively. For the victim drug with no renal clearance (i.e., \( f_{renal} = 0 \), eq. 2 is reduced to eq. 3, which is commonly used to describe P450-mediated DDI predictions (Obach et al., 2006):

\[ AUCR = \frac{Fa'}{Fa} \times \frac{Fg'}{Fg} \times \left( \frac{CL_{int,h} + CL_{sec}}{CL_{sec}} \right) \times \left( 1 - \frac{\sum fm_{CYP}}{1 - \sum fm_{CYP}} \right) \]  

(3)

\( RL_{CYP} \) is the competitive inhibition term (eq. 6), \( TDICYP \) is the time-dependent inhibition term (eq. 7) against hepatic P450 enzymes, and \( RL_{OAT3} \) is the competitive inhibition term (eq. 6) against renal basolateral transporter OAT3.

Extended Net-Effect Model Accounting for Transporter-Enzyme Interplay. For victim drugs that are subjected to active hepatic uptake (e.g., statins and glinides), \( CL_{int,h} \) is mathematically defined by the extended clearance concept (Liu and Pang, 2005; Shitara et al., 2006; Shitara and Sugiyama, 2006; Camenisch and Umehara, 2012; Barton et al., 2013; Li et al., 2014):
PS\textsubscript{active} and PS\textsubscript{pd} are sinusoidal active uptake clearance and passive diffusion, respectively. Active uptake was assumed to be primarily OATP1B1-mediated transport (basolateral active efflux is not considered). CL\textsubscript{int,h} is biliary intrinsic clearance. \( \sum \text{CL}_{\text{int,CYP}} \) represents the sum of intrinsic metabolic clearances by individual CYP enzymes and can also be expressed as: \( \text{CL}_{\text{int,met}} = \text{CL}_{\text{int,CYP}} + \text{CL}_{\text{int,OATP}} \). SF\textsubscript{active} represents the empirical scaling factor for active uptake estimated by matching the in vitro CL\textsubscript{int,h} (eq. 4) to the in vivo CL\textsubscript{int,h} obtained from intravenous pharmacokinetics (eq. 11). The geometric mean SF\textsubscript{active} of 10.6, established by utilizing sandwich-cultured human primary hepatocytes (three hepatocyte lots) for 10 different OATP substrates, was applied (Varma et al., 2014). The in vitro intrinsic values were scaled assuming the following: 118 × 10\(^6\) hepatocytes g\(^{-1}\) liver, 39.8 mg microsomal protein g\(^{-1}\) liver, and 24.5 g liver kg\(^{-1}\) body weight (Varma et al., 2013b).

In the presence of perpetrator, the expected net-effect of reversible inhibition, time-dependent inhibition and induction, can be represented by

\[
\text{CL}_{\text{int,h}} = \left( \text{SF}_{\text{active}} \times \text{PS}_{\text{active}} + \text{PS}_{\text{pd}} \right) \left( \frac{\left( \sum \text{CL}_{\text{int,CYP}} + \text{CL}_{\text{int,h}} \right)}{\left( \text{PS}_{\text{pd}} + \sum \text{CL}_{\text{int,CYP}} + \text{CL}_{\text{int,h}} \right)} \right)
\]

for binding to microsomal protein (6.9 \text{ h}^{-1}) for hepatic CYP2C8 (Fahmi et al., 2008; Lai et al., 2009).

Changes in active renal secretion (CL\textsubscript{\text{ur,Rb}}) caused by inhibition of OAT3 by gefitinib is described by a basic model (Feng et al., 2013):

\[
\text{CL}_{\text{ur,Rb}} = \left( \frac{1 + \frac{I_u}{C_{2,Rb}}} {1 + \frac{I_u}{C_{2,RB}}} \right) \frac{Q_{\text{h}}}{F_{\text{gut}}}
\]

where CL\textsubscript{\text{ur}} \(= (\text{CL}_{\text{ur}} - \text{CL}_{\text{ur}})\text{Rb}\) is the hepatic blood clearance obtained from intravenous total plasma clearance corrected for renal clearance and blood-to-plasma ratio (\( R_b \)).

**PBPK Modeling and Simulations**

Modeling and simulations were performed using the population-based absorption, distribution, metabolism, and excretion (ADME) simulator Simcyp (version 13; Simcyp Ltd., Sheffield, United Kingdom). Each simulation was performed for 50 virtual populations of healthy subjects (5 trials × 10 subjects). The PBPK models for gefitinib and Gem-Glu were similar to those described elsewhere (Varma et al., 2012; Barton et al., 2013). Briefly, a model for gefitinib was constructed using the clinical first-order absorption rate (\( F_a \)) observed volume of distribution (0.08 l/kg) and oral clearance (6.0 l/h) (Table 1). Based on the in vitro data, the model considered 79% of gefitinib dose underwent glucuronidation to form Gem-Glu, and the rest was accounted for by CYP3A-dependent metabolism (Kilford et al., 2009). A lag-time of 15 minutes was considered for oral absorption based on the oral pharmacokinetic profiles (Varma et al., 2012). A model for Gem-Glu was built based on metabolite formation rate from gefitinib, \( f_{\text{g}} \), and the blood/plasma ratio. Due to the lack of disposition data for the metabolite, the \( V_{\text{g}} \) (0.1 l/kg) and biliary intrinsic clearance (5.2 \text{ l/min/100g}) for the metabolite were estimated (assuming complete elimination via bile) by “retrograde” fitting to the observed plasma concentration-time profile, assuming perfusion-limited disposition.

The following interaction parameters were applied in the PBPK model: gefitinib \( \text{K}_{\text{OATP}} \) (2.54 \text{ l/min/106 cells}) for the metabolite were estimated using the Rodgers and Rowland (2006) method considering rapid equilibrium transport. Models for the OATP1B1 substrates, cerivastatin and repaglinide, were built using the approach similar to that described earlier (Varma et al., 2012; Barton et al., 2013; Jamei et al., 2014). A full-PBPK model using the Rodgers and Rowland (2006) method considering rapid equilibrium between liver and blood compartments using the physicochemical properties and in vitro preclinical data such as human plasma \( f_{\text{u}} \), blood-to-plasma ratio, and metabolic intrinsic clearance values (Table 1). In the case of rosiglitazone, the model (also assumed rapid equilibrium) was adopted from Simcyp V13 compound files. Models for the OATP1B1 substrates, cerivastatin and repaglinide, were built using the approach similar to that described earlier (Varma et al., 2012; Barton et al., 2013; Jamei et al., 2014). A full-PBPK model using the Rodgers and Rowland (2006) method considering rapid equilibrium between blood and tissues was adopted to obtain the distribution into all organs except the liver. Permeability-limiting distribution into liver was considered, for which sinusoidal active uptake intrinsic clearance and passive diffusion obtained from sandwich-cultured human hepatocytes studies were incorporated to capture hepatic disposition. The intrinsic transport clearance values were adopted from an earlier report (Varma et al., 2014). A scaling factor for the active uptake intrinsic clearance, which was initially assumed as unity, was estimated by a “top-down” approach of fitting to the intravenous plasma.
### TABLE 1

Summary of victim drug input parameters for PBPK modeling and simulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pioglitazone$^a$</th>
<th>Rosiglitazone$^b$</th>
<th>Repaglinide$^c$</th>
<th>Cerivastatin$^d$</th>
<th>Gemfibrozil$^e$</th>
<th>Gemfibrozil-1-O-β-Glucuronide$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physicochemical properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>356.4</td>
<td>357.4</td>
<td>452.6</td>
<td>459</td>
<td>250.3</td>
<td>426.5</td>
</tr>
<tr>
<td>Log P</td>
<td>3.5</td>
<td>2.6</td>
<td>4.87</td>
<td>1.8</td>
<td>4.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Compound type</td>
<td>Monoprotic base</td>
<td>Ampholyte</td>
<td>Monoprotic acid</td>
<td>Monoprotic acid</td>
<td>Monoprotic acid</td>
<td>Monoprotic acid</td>
</tr>
<tr>
<td>pK_a</td>
<td>5.53</td>
<td>6.25 and 6.32</td>
<td>4.19 and 5.78</td>
<td>5</td>
<td>4.75</td>
<td>2.68</td>
</tr>
<tr>
<td>Fraction unbound</td>
<td>0.015</td>
<td>0.002</td>
<td>0.015</td>
<td>0.013</td>
<td>0.008</td>
<td>0.115</td>
</tr>
<tr>
<td>Blood/plasma ratio</td>
<td>1</td>
<td>0.57</td>
<td>0.62</td>
<td>0.76</td>
<td>0.825</td>
<td>0.825</td>
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<tr>
<td><strong>Absorption</strong></td>
<td></td>
<td></td>
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<tr>
<td>Absorption type</td>
<td>ADAM</td>
<td>1st order</td>
<td>ADAM</td>
<td>ADAM</td>
<td>1st order</td>
<td></td>
</tr>
<tr>
<td>Fraction absorbed</td>
<td>0.98</td>
<td>1 ($K_a = 3.6 \text{ h}^{-1}$)</td>
<td>0.99</td>
<td>0.75</td>
<td>1 ($K_a = 3 \text{ h}^{-1}$)</td>
<td></td>
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<tr>
<td>Caco-2 permeability (×10^{-6} cm/s)</td>
<td>31</td>
<td>26.1</td>
<td>10</td>
<td>1.873</td>
<td>1.873</td>
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<tr>
<td>Absorption scalar</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$f_{in}$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Distribution</strong></td>
<td></td>
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</tr>
<tr>
<td>Distribution model</td>
<td>Minimal PBPK</td>
<td>Minimal PBPK</td>
<td>Full PBPK (method 2)</td>
<td>Full PBPK (method 2) ($K_p$ scalar 1.5)</td>
<td>Minimal PBPK</td>
<td>Minimal PBPK</td>
</tr>
<tr>
<td>$V_v$ (l/kg)</td>
<td>0.253</td>
<td>0.12</td>
<td>0.256</td>
<td>0.31</td>
<td>0.08</td>
<td>0.1</td>
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<td><strong>Elimination</strong></td>
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<tr>
<td>$\text{Cl_{int,CYP2C8}}$ (μl/min/mg)</td>
<td>27.5 (HLM)</td>
<td>191 (HLM)</td>
<td>93 (HLM)</td>
<td>19.1 (HLM)</td>
<td>0.29 (CYP)</td>
<td></td>
</tr>
<tr>
<td>$\text{Cl_{int,CYP3A}}$ (μl/min/mg)</td>
<td>1.5 (HLM)</td>
<td>38 (HLM)</td>
<td>12.7 (HLM)</td>
<td>0.7</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Biliary $\text{Cl_{int}}$ (μl/min/10^{6} cells)</td>
<td>6.1 (CYP2C19-HLM)</td>
<td>102 (CYP2C9-HLM)</td>
<td>65 (UGT2B7-HLM)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Renal clearance (l/h)</td>
<td>0.32</td>
<td></td>
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<tr>
<td><strong>Hepatobiliary transport</strong></td>
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<tr>
<td>Liver unbound fraction (Intra-extracellular)</td>
<td>0.143/0.028</td>
<td>0.97/0.025</td>
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<td></td>
</tr>
<tr>
<td>Passive diffusion (μl/min/10^{6} cells)</td>
<td>22</td>
<td>17.5</td>
<td></td>
<td></td>
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<tr>
<td>$\text{Cl_{int,fac}}$ (μl/min/10^{6} cells)</td>
<td>35.5</td>
<td>16.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scaling factor (active uptake)</td>
<td>18.7$^g$</td>
<td>30.5$^g$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>References</td>
<td>(Jaakkola et al., 2006)</td>
<td>(Varma et al., 2013a)</td>
<td>(Muck et al., 1997; Varma et al., 2014)</td>
<td>(Varma et al., 2013a)</td>
<td>(Varma et al., 2013a)</td>
<td>(Varma et al., 2013a)</td>
</tr>
</tbody>
</table>

ADAM, advanced dissolution, absorption, and metabolism model; $P$, partition coefficient; $pK_a$, acid dissociation constant.

$^a$ Models developed in this current study based on the literature in vitro data (pioglitazone) or in-house in vitro data (cerivastatin).

$^b$ Pioglitazone model was adopted from Simcyp V13 compound files.

$^c$ Model adopted from a previous report (Barton et al., 2013).

$^d$ Model modified from a previous report (Barton et al., 2013).

$^e$ Gemfibrozil model was adopted from Simcyp V13 compound files.

$^f$ Model modified from a previous report (Barton et al., 2013).

$^g$ Estimated by fitting to intravenous data. See Materials and Methods.
Results

Prediction of Gemfibrozil Interactions Using Static Models. Two mechanistic static models were used to predict gemfibrozil DDIs depending upon the disposition characteristics of the victim drugs: a net-effect model (Fahmi et al., 2008), assuming rapid equilibrium between liver and blood compartments for victim drug (not substrate to OATPs), and an extended net-effect model (Varma et al., 2013b, 2014), considering the extended clearance concept for the victim drug (OATPs substrate). Assuming inhibition of hepatic OATP1B1 and CYP2C8 and renal OAT3 by both gemfibrozil and Gem-Glu, the AUCR in 75% of cases was predicted within 1.5-fold of the clinically observed AUCRs; 98% were predicted within 2-fold of the observed AUCRs (Fig. 1). Furthermore, on the basis of bioequivalence limits, only three false-positive (predicted >1.25-fold, observed ≤1.25-fold) and three false-negative (predicted ≤1.25-fold, observed >1.25-fold) predictions were noted among the 48 DDIs assessed. Imatinib, zopiclone, and (S)-ibuprofen were false-negative predictions, although the predicted AUCRs are below 1.5.

The three false-negative predictions involved simvastatin, ezetimibe, and empagliflozin, which are suggested to possess some degree of hepatic active uptake transport (Oswald et al., 2008; Macha et al., 2014)—not captured in the current assessment due to limited transporter data.

Inhibition of OAT3-mediated renal secretion by the parent and metabolite contributed significantly to the predicted AUCR of sitagliptin, pravastatin, and rosuvastatin (Table 2). However, such an effect was not seen with other victim drugs due to their minimal renal secretion.

When combined with gemfibrozil, itraconazole (a potent CYP3A inhibitor) increased the AUCR of victim drugs including repaglinide and loperamide. Static models predicted these complex effects within 1.5-fold of the observed values. Clearly, parent (gemfibrozil) alone significantly underpredicted the AUCR (Fig. 1A), whereas inclusion of the metabolite (Gem-Glu) in the static models recovered the observed AUCRs (Fig. 1B).

PBPK Modeling and Quantitative DDI Predictions. A PBPK model was developed based on the data in Table 1, which described the plasma concentration-time profiles of gemfibrozil and Gem-Glu (Fig. 2). On the basis of in vitro data, model predictions suggest that the presence of Gem-Glu almost completely inhibited CYP2C8 activity after the first dose of 600 mg of gemfibrozil. Moreover, consideration of Gem-Glu increased the inhibition of OATP1B1 (Fig. 2C).

PBPK models for the victim drugs not subjected to active hepatic uptake, pioglitazone and rosiglitazone, were developed assuming rapid equilibrium between blood and all tissue compartments (Table 1). However, permeability-limited hepatic disposition was assumed for the OATP1B1 and CYP2C8/3A4 dual substrates repaglinide and cerivastatin. Models were constructed using the in silico, in vitro, and intravenous pharmacokinetic data and were further verified using the oral pharmacokinetic profiles (Fig. 3). The model predictions of pioglitazone-gemfibrozil interaction are similar to those observed in the clinic when considering TDI by Gem-Glu, without which no significant AUC change was predicted (Fig. 3A). Similarly, in the case of rosiglitazone interactions, the parent alone predicted no AUC change (<1.25-fold) whereas incorporation of Gem-Glu in the predictions recovered the observed AUC ratio (Fig. 3B). Repaglinide and cerivastatin interactions with gemfibrozil are well recovered when both parent and metabolite are considered (Fig. 3, C and D). Gemfibrozil alone showed weak DDI (<1.5-fold) for the OATP1B1 substrates repaglinide and cerivastatin.

A sensitivity analysis on the impact of metabolite-to-parent exposure on gemfibrozil DDIs suggested that less than 10% of the circulating Gem-Glu after 600 mg of gemfibrozil is enough to move the DDI category from weak or no interaction (<2-fold) to a moderate risk category (2- to 5-fold) (Fig. 4). Change in pioglitazone and rosiglitazone AUC was minimal above the metabolite-to-parent ratio of 0.1, whereas a significant increase in the cerivastatin and repaglinide AUC was predicted as the metabolite-to-parent ratio increase due to competitive inhibition of OATP1B1-mediated hepatic uptake.

Discussion

Gemfibrozil is a widely prescribed drug and is also the recommended inhibitor to probe the role of CYP2C8 in the clearance...
<table>
<thead>
<tr>
<th>Static Model</th>
<th>Victim Drug</th>
<th>Gemfibrozil Dose</th>
<th>$f_{\text{MCYP2C8}}$</th>
<th>$F_{\text{Basal}}$</th>
<th>$F_{\text{sec}}$</th>
<th>Observed AUCR</th>
<th>Predicted (Gemfibrozil Only)</th>
<th>Predicted (Gemfibrozil and Gem-Glu)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>(S)-warfarin</td>
<td>600 mg (8 d)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0 (Kaminsky and Zhang, 1997; Lilja et al., 2005)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>Lovastatin</td>
<td>600 mg (3 d)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>(Wang et al., 1991; Chen et al., 2012)</td>
<td></td>
<td></td>
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<tr>
<td>NE</td>
<td>Imatinib</td>
<td>600 mg (6 d)</td>
<td>0.25</td>
<td>0.05</td>
<td>0.46</td>
<td>0.9</td>
<td>1.1</td>
<td>1.3 (Nebo et al., 2010; Filippa et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>(R)-warfarin</td>
<td>600 mg (8 d)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0 (Kaminsky and Zhang, 1997; Lilja et al., 2005)</td>
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<tr>
<td>NE</td>
<td>Brivaracetam</td>
<td>600 mg (7 d)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>(Nicolás et al., 2012)</td>
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<tr>
<td>NE</td>
<td>Lovastatin</td>
<td>600 mg (3 d)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Wang et al., 1991; Kyrklund et al., 2001)</td>
<td></td>
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<tr>
<td>NE</td>
<td>Zopiclone</td>
<td>600 mg (3 d)</td>
<td>0.3</td>
<td>0.03</td>
<td>0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4 (Beccaaemont et al., 1999; Tarno et al., 2006)</td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>Zafirlukast</td>
<td>600 mg (5 d)</td>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Dekhuijzen and Koopmans, 2002; Karonen et al., 2011)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**References**

- Kaminsky and Zhang, 1997
- Lilja et al., 2005
- Wang et al., 1991
- Chen et al., 2012
- Nebo et al., 2010
- Filippa et al., 2013
- Nicolás et al., 2012
- Kyrklund et al., 2001
- Beccaaemont et al., 1999
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- Wang et al., 1991
- Kyrklund et al., 2001
- Beccaaemont et al., 1999
- Tarno et al., 2006
- Dekhuijzen and Koopmans, 2002
- Karonen et al., 2011

**Notes:**

- ENE: extended net-effect model; Itra: itraconazole; NE: net effect model.
- Value in parentheses indicates $f_{\text{MCYP3A4}}$.

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**TABLE 2**

Summary of victim-gemfibrozil DDI predictions using mechanistic static models.
of investigational drugs. Here we attempted to rationalize a wide array of clinical DDIs of gemfibrozil using a set of 48 data points, so as to, 1) implement the learnings for the prospective predictions of gemfibrozil interactions early in drug development and strategize the clinical studies, 2) understand the quantitative role of a major circulating metabolite in governing the magnitude of the DDI, and 3) assess the ability of mechanistic static and dynamic PBPK models to predict complex drug interactions involving transporter-enzyme interplay and multiple inhibitory species (parent and metabolite).

Gemfibrozil and its metabolite, Gem-Glu, can bring about DDIs by multiple mechanisms, which include CYP2C8, OATP1B1, and OAT3 inhibition. These mechanisms are simultaneously implemented in the mechanistic models to predict the DDIs of victim drugs with a wide range of disposition characteristics. For a dataset of 48 DDIs, the static models predicted 75% and 98% of the cases within 1.5- and 2-fold of the observed AUCR values (Fig. 1). Similarly, for representative drugs (pioglitazone, rosiglitazone, cerivastatin, and repaglinide), PBPK modeling and simulations well recovered the plasma concentration-time profiles in control and gemfibrozil treatment conditions (Fig. 3).

It was obvious that the inclusion of Gem-Glu in the static and PBPK models greatly improved the accuracy of the predictions—consideration of parent only led to a significant underprediction of AUCR (Fig. 1 and 3). Furthermore, model predictions suggest that Gem-Glu has a much larger inhibitory effect than that of the parent in vivo (Fig. 4).

Repaglinide and cerivastatin are mainly metabolized by CYP2C8 and CYP3A4 to their respective major oxidative metabolites, and they are subjected to OATP1B1-mediated sinusoidal uptake also (Kajosaari et al., 2005b; Niemi et al., 2005; Jones et al., 2012). Consequently, one would have to anticipate complex DDIs when these drugs are dosed with perpetrators impacting one or more of these processes. We have characterized the hepatic transport of repaglinide and cerivastatin using sandwich-cultured human hepatocytes and have developed mechanistic models incorporating transporter-enzyme interplay. In terms of extended clearance concept (Shitara et al., 2006; Li et al., 2014), the total hepatic uptake for repaglinide and cerivastatin is significantly higher than the passive uptake, and the passive uptake and metabolic intrinsic clearance are within a similar range (Table 1). Therefore, the systemic clearance of these drugs decreases by inhibiting active uptake or metabolism, with a much larger impact caused by simultaneous inhibition of both processes (Varma et al., 2015).

Earlier studies suggested an in vitro–in vivo disconnect in the repaglinide $f_{\text{mCYP2C8}}$ on the basis of a collection of clinical repaglinide-gemfibrozil DDI reports (Bidstrup et al., 2003; Kajosaari et al., 2005a; Honkalammi et al., 2011a, 2012). However, with the current mechanistic models (extended net-effect and PBPK models) accounting for transporter-enzyme interplay, repaglinide-gemfibrozil interactions are closely predicted using $f_{\text{mCYP2C8}}$ determined in vitro. Repaglinide and cerivastatin share similar ADME characteristics: both are highly permeable OATP1B1 and CYP2C8/3A4 substrates with intrinsic transport and metabolic clearances in a similar range (Table 1). With the static and PBPK models, the cerivastatin-gemfibrozil interaction of $\sim$5-fold can be recovered with in vitro $f_{\text{mCYP2C8}}$ of only 0.65 (Fig. 3). Overall, consistent with the in vitro observations, both CYP2C8 and CYP3A contribute to the metabolism
of repaglinide and cerivastatin in vivo, while the larger than expected clinical interaction with gemfibrozil is due to simultaneous inhibition of both OATP1B1-mediated active uptake and CYP2C8-based metabolism.

Recent regulatory guidelines have suggested the use of repaglinide as a clinical probe for the assessment of CYP2C8 and OATP1B1 inhibition (CDER, 2012; CHMP, 2012). However, based on the in vitro findings and the mechanistic evaluation, in vivo fmCYP2C8 could be smaller than previously thought, and repaglinide may not be an ideal substrate to probe CYP2C8 inhibition in vivo. It is expected that a potent OATP1B1 inhibitor or potent CYP3A and OATP1B1 inhibitor combination can also result in a comparable increase in repaglinide AUC (Varma et al., 2013a). On the other hand, investigational drugs with moderate reversible inhibition of CYP2C8 and no OATP1B1 inhibition will likely precipitate weak or no clinical DDI with repaglinide as the victim drug. Due to the lack of alternative sensitive probes, CYP2C8 substrates with no active hepatic uptake that show an observed and predicted DDI in the moderate to high range, such as pioglitazone and montelukast, should be considered for clinical studies (Table 2).

One of the aims of this study was to determine the quantitative contribution of the major metabolite(s) to the in vivo DDIs, and to examine the application of the regulatory recommendations to trigger in vitro investigation of the interaction potential of metabolites when present at \( \geq 25\% \) of the parent AUC. Sensitivity analysis on the impact of the metabolite-to-parent ratio on AUCR suggested that Gem-Glu exposure at \( \geq 10\% \) of the parent moved the DDI category from no or weak (AUCR <2) to moderate (2 < AUCR <5) for all four victim drugs (Fig. 4). This suggests that the Gem-Glu exposure needed to bring about complete inactivation of CYP2C8 is only a fraction of that achieved in the clinic after a 600-mg gemfibrozil dose. However, due to moderate OATP1B1 inhibition by the metabolite, an increase in the metabolite-to-parent ratio further increased the AUCR for repaglinide and cerivastatin (Fig. 4, C and D). These model predictions are consistent with clinical findings where a subtherapeutic gemfibrozil dose (30 mg; 20 times lower than therapeutic dose) caused a \( \sim 3.4\)-fold increase in repaglinide AUC while a further increase in AUCR was noted with higher doses (100-mg and 600-mg gemfibrozil doses yielded repaglinide AUCR of \( \sim 5.5 \) and \( \sim 7 \), respectively) (Honkalammi et al., 2012). Overall, the default metabolite-to-parent exposure cutoff (\( \geq 25\% \)) may not firmly reflect the DDI potential of gemfibrozil due to the CYP2C8 TDI component. Furthermore, the DDI risk in vivo when metabolites inhibit both uptake transport and metabolism is expected to be large. So the early risk assessment of metabolite-mediated DDIs should consider structural alerts for TDI, in vitro interaction potential against enzymes and transporters, and systemic exposure of both parent and metabolites (Callegari et al., 2013; Yu et al., 2015). The resulting data can then be integrated in the static or PBPK models for DDI predictions, as described here.

Gemfibrozil has been proposed as strong inhibitor to assess the role of CYP2C8 in the clinical pharmacokinetics of the investigational drugs (CDER, 2012; CHMP, 2012). However, due to its multiple interaction mechanisms, the interpretation of the clinical study data will be challenging for investigational drugs that serve as OATP1B1 substrates also. Our predictions suggest the use of subtherapeutic doses of gemfibrozil (100 mg twice a day) in such situations, where the competitive inhibition of transporters is insignificant but the CYP2C8 inactivation is almost complete (Figs. 2D and 4).
The CYP2C8 inactivation mechanism of Gem-Glu (phase II metabolite) has focused some attention on acyl-glucuronides as TDIs of P450s, particularly CYP2C8. To date, however, inhibition of CYP2C8 has been observed only with Gem-Glu, and more recently with clopidogrel-acyl-β-D-glucuronide (Jenkins et al., 2011; Tornio et al., 2014). On the other hand, ezetimibe glucuronide, with exposure ~4 times that of the ezetimibe, has been shown to inhibit OATPs in vitro; however, no DDI with statins is evident (Patino-Rodriguez et al., 2014). Beyond Gem-Glu, only clopidogrel-acyl-β-D-glucuronide has shown similar interaction mechanisms manifesting in clinically relevant DDI. In this instance, it has been noted that the incidence of cerivastatin-induced rhabdomyolysis is higher (odds ratio of ~30) in clopidogrel users (Floyd et al., 2012), potentially due to a ~5-fold increase in cerivastatin exposure as a result of CYP2C8 inactivation and competitive inhibition of OATP1B1 by clopidogrel and its metabolites including clopidogrel-acyl-β-D-glucuronide (Tornio et al., 2014). Further investigation is warranted in identifying the structural attributes of gemfibrozil and clopidogrel acyl-glucuronides that render CYP2C8 inactivation—which could flag probable glucuronides for early in vitro DDI assessment.

Our study reinforces the utility of in vitro data and the modeling approaches that mechanistically integrate the multiple components in the DDI risk assessment. The importance of understanding transporter-enzyme interplay and the role of perpetrator metabolites needs to be emphasized. In a true prospective sense, it is challenging to predict plasma concentrations of the metabolites at the discovery stage due to limited in vitro information on their disposition and/or species differences in parent/metabolite handling. Moreover, the disposition of phase II metabolites like acyl-glucuronides is primarily determined by hepatic and renal transporters, which further complicate pharmacokinetic predictions. We therefore used the observed plasma concentration data of Gem-Glu in the static models and in the process of building the metabolite PBPK model. Early predictions that consider the sensitivity analysis of uncertain parameters, particularly for metabolite exposure, could be helpful at the drug discovery stage, but there is need to refine the models of both parent and metabolite(s) as the clinical pharmacokinetic and human ADME data become available. The mechanistic static models described here will be valuable in the early discovery and development stage, and the PBPK models can be applied for more refined predictions based on the initial clinical data. Importantly, once developed and verified, such static and PBPK models can be used to project DDIs for new victim drugs, support risk assessment, and inform clinical DDI study planning and prioritization.

In summary, the complex DDIs involving perpetrator parent/metabolite pair and multiple inhibitory mechanisms (transporters and enzymes) can be quantitatively rationalized by informing mechanistic static and PBPK models with thoroughly characterized interaction parameters of the perpetrator species and the disposition attributes (transporter-enzyme interplay) of the victim drug. The mechanistic static and PBPK model developed and validated leveraging a large clinical dataset should provide confidence in the DDI risk assessment involving gemfibrozil coadministration, and potentially avoid unnecessary clinical studies.
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Authorship Contributions
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Performed data analysis: Varma, Lin.
Wrote or contributed to the writing of the manuscript: Varma, Lin, Bi, Kimoto, Rodrigues.

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