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

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**Abbreviations:** AUC, area under the plasma concentration-time curve; AUCR, area under the plasma concentration-time curve ratio; CYP, cytochrome P-450; CL_{int,bile}, biliary intrinsic clearance; CL_{int,met}, intrinsic metabolic clearance; CL_{int,h}, intrinsic hepatic clearance; CL_{h}, hepatic clearance; CL_{r}, renal clearance; DDI, drug-drug interactions; Fa, fraction absorbed; Fg, fraction of drug escaping gut-wall extraction; Fh, fraction of drug escaping hepatic extraction; f_{u,gut}, fraction unbound in the gut; Gem-Glu, gemfibrozil 1-O-β-glucuronide; I_{u,max}, maximum unbound plasma perpetrator concentration; I_{u,max,in}, maximum unbound perpetrator concentration at the inlet to liver; Ka, absorption rate constant; k_{deg,h}, apparent first-order degradation rate constant; Ki, inhibition constant; k_{inact}, maximal inactivation rate constant; K_I, inhibitor concentration that supports half the maximal rate of inactivation; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; PBPK, physiologically-based pharmacokinetic; PS_{active}, active uptake clearance; PS_{pd}, passive diffusion; Q_{gut}, enterocytic blood flow; Rb, blood-to-plasma ratio; SCHH, sandwich cultured human hepatocytes; SF_{active}, scaling factor for active uptake; TDI, time-dependent inhibition; V_{ss}, volume at steady-state.
ABSTRACT

Gemfibrozil has been suggested as a sensitive cytochrome P-450 (CYP)2C8 inhibitor for clinical investigation by the US Food and Drug Administration and the European Medicines Agency. However, gemfibrozil drug-drug interactions (DDIs) are complex as its major circulating metabolite, gemfibrozil 1-O-β-glucuronide (Gem-Glu), exhibits time-dependent inhibition of CYP2C8 and both parent and metabolite also behave as moderate inhibitors of organic anion transporting polypeptide (OATP)1B1 in vitro. Additionally, parent and metabolite also inhibit renal transport mediated by organic anion transporter 3. Here, in vitro inhibition data for gemfibrozil and Gem-Glu were utilized to assess 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. Of 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-β-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 following 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.
INTRODUCTION

Drug-drug interactions (DDIs) may significantly alter drug exposure and subsequently influence the efficacy and safety profiles of a drug. Therefore, the ability to quantitatively predict DDIs, primarily involving drug metabolizing enzymes and transporters, is essential for risk assessment at drug discovery and early development stages. However, drug interactions may be mechanistically complex due to inhibition and/or induction of multiple enzymes and/or transporters, concomitant use of multiple perpetrator drugs and the presence of perpetrator metabolites (Sager et al., 2014; Varma et al., 2015). Considerable attention has been drawn towards the contribution of perpetrator metabolites to DDIs due to inhibition of enzymes and transporters (Isoherranen et al., 2009; Yeung et al., 2011; Zamek-Gliszczynski et al., 2014). Current European Medical Agency (EMA) and US Food and Drug Administration (USFDA) guidelines on DDIs recommend \textit{in vitro} investigation of metabolite’s interaction potential if present at \(\geq 25\%\) of the parent area under the plasma concentration-time curve (AUC) (USFDA, 2012) or \(\geq 25\%\) of the parent AUC and \(\geq 10\%\) of the total drug-related AUC (EMA, 2012). Under the auspices of the Drug Metabolism Leadership Group of the Innovative and Quality Consortium (IQ-DMLG), the Metabolite-mediated DDI Scholarship Group (MDSG) – tasked to examine the application of these regulatory recommendations – was formed with scientist representing 18 pharmaceutical companies (Yu et al., 2015). Based on the analysis of 137 frequently prescribed drugs, the MDSG concluded that the risk for unexpected clinical DDI as a result of not assessing \textit{in vitro} cytochrome P450 (P450) inhibition by metabolite(s) is low; which is consistent with earlier reports (Yeung et al., 2011; Callegari et al., 2013). However, five of the 137 perpetrator drugs (amiodarone, buproprion, gemfibrozil, sertraline and capecitabine) were noted false negative predictions (parent \textit{in vivo} inhibition negative, but \textit{in vivo} inhibition
positive), and showed evidences that metabolites contribute to the observed \textit{in vivo} P450-based DDIs. To further investigate these perpetrator drugs and assess the quantitative contribution of the circulating metabolite(s), a Metabolite Scholarship PBPK Subteam was formed and focused 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 (VLDL) triglyceride and high density lipoprotein (HDL) cholesterol levels, in all types of dyslipidaemia (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 (Niemi 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-\(O\)-\(\beta\)-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 that had died were also taking gemfibrozil along with cerivastatin. Subsequently, several studies were conducted to understand the mechanisms involved, and it was determined that gemfibrozil (b.i.d 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) (Niemi et al., 2003b; Honkalammi et al., 2011a) pioglitazone (~4-fold increase in AUC) (Aquilante et al., 2013), and rosiglitazone (~2.5-fold increase in AUC) (Niemi et al., 2003a). The results of complimentary \textit{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.

There is rich clinical data with several DDI studies reported for gemfibrozil, which is now suggested as the clinical probe inhibitor for CYP2C8 activity by the USFDA, the EMA and other agencies (EMA, 2012; USFDA, 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 prior to human dosing and enable subsequent clinical study planning and (2) assess the contribution of the circulating metabolite to the magnitude of DDIs. By leverage information from 48 different clinical DDI studies reported for gemfibrozil, in conjunction with available in vitro inhibition data for parent and metabolite, it was possible to employ both static mechanistic and dynamic physiologically-based pharmacokinetic (PBPK) modelling 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 scientific literature.

Static Model Predictions

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

\[
AUCR = \frac{\text{Fa}' \cdot \text{Fg}' \cdot \text{Fh}' \cdot (\text{CL}_b + \text{CL}_r)}{\text{Fa} \cdot \text{Fg} \cdot \text{Fh} \cdot (\text{CL}_b' + \text{CL}_r')}
\]

Where,

\[
\text{Fh} = 1 - \frac{\text{CL}_b}{Q_h}
\]

\[
\text{CL}_b = \frac{Q_h \cdot f_{u,b} \cdot \text{CL}_{\text{int},b}}{Q_h + f_{u,b} \cdot \text{CL}_{\text{int},b}}
\]

\[
\text{CL}_r = f_{u,r} \cdot \text{GFR} + \text{CL}_{\text{sec}}
\]

Fa, Fg, Fh represent the fraction of drug absorbed, fraction of drug escaping gut-wall extraction and hepatic extraction, respectively. Fa`, Fg`, Fh` are corresponding parameters in the presence
of perpetrator. CL\textsubscript{L}, CL\textsubscript{R}, CL\textsubscript{L'} and CL\textsubscript{R'} represent hepatic and renal blood clearance in the absence and presence of the perpetrator, respectively. CL\textsubscript{int,L} is intrinsic hepatic clearance, f\textsubscript{u,L} is fraction unbound in blood, and Q\textsubscript{h} is 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 glomerular filtration rate (GFR, 1.78 mL/min/kg) and active secretion (CL\textsubscript{sec}).

**Net-Effect Model.** Gemfibrozil interactions occur primarily at the level of liver (OATP1B1 and CYP2C8 inhibition) and kidney (OAT3 inhibition). Therefore, for victim drugs not subjected to active hepatic uptake, AUC\textsubscript{R} was predicted using the static “net-effect” model.

\[
AUC\textsubscript{R} = \frac{\frac{F_a'}{F_a} \cdot \frac{F_g'}{F_g} \cdot \frac{1}{f_{\text{Hepatic}} \cdot \left( \sum f_{\text{CYP}} \cdot \frac{RI_{\text{CYP}} \cdot TDI_{\text{CYP}}}{1 - \sum f_{\text{CYP}}} \right) + f_{\text{Renal}} \left( \frac{f_{\text{sec}}}{RI_{\text{OAT3}}} + f_{\text{GFR}} \right)}}{f_{\text{Renal}} + f_{\text{Hepatic}}}
\]  

Where, \( f_{\text{Hepatic}} + f_{\text{Renal}} = 1 \)

\[
\sum f_{\text{CYP}} + \left( 1 - \sum f_{\text{CYP}} \right) = 1
\]

and \( f_{\text{sec}} + f_{\text{GFR}} = 1 \)

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

\[
AUCR = \frac{F_{a}'}{F_{a}} \cdot \frac{F_{g}'}{F_{g}} \cdot \frac{1}{\sum R_{I_{CYP}} \cdot T_{D I_{CYP}}} + (1 - \sum f_{m_{CYP}})
\]  

(3)

\(RI_{CYP}\) is the competitive inhibition term (Eq. 6), \(TDI_{CYP}\) is the time-dependent inhibition term (Eq. 7) against hepatic CYP enzymes. \(RI_{OAT3}\) 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).

\[
CL'_{int,h} = \left( SF_{active} \cdot PS_{active} + PS_{pd} \right) \left( \frac{\sum CL_{int,CYP} + CL_{int,bile}}{PS_{pd} + \sum CL_{int,CYP} + CL'_{int,bile}} \right)
\]  

(4)

\(PS_{active}\) and \(PS_{pd}\) 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). \(CL_{int,bile}\) is biliary intrinsic clearance. \(\sum CL_{int,CYP}\) represents the sum of intrinsic metabolic clearances by individual CYPs, and can also be expressed as: \(CL_{int,met}.f_{m_{CYP-X}} + CL_{int,met}.(1-f_{m_{CYP-X}})\), where \(CL_{int,met}\) is the total intrinsic metabolic clearance. \(SF_{active}\) represents empirical scaling factor for active uptake estimated by matching the \textit{in vitro} \(CL_{int,h}\) (Eq. 4) to the \textit{in vivo} \(CL_{int,h}\), obtained from intravenous pharmacokinetics (Eq. 11).
geometric mean \( SF_{active} \) of 10.6, established utilizing sandwich cultured human primary hepatocytes (SCHH; three hepatocyte lots) for 10 different OATP substrates, was applied (Varma et al., 2014). The \textit{in vitro} intrinsic values were scaled assuming the following: \(118 \times 10^6\) hepatocytes g\(^{-1}\) liver, 39.8 mg microsomal protein g\(^{-1}\) liver, 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,

\[
\text{CL}_{int,h} = \left( \frac{\text{SF}_{active} \cdot \text{PS}_{active}}{\text{RI}_{OATP}} + \text{PS}_{pd} \right) \cdot \frac{\left( \sum \frac{\text{CL}_{int,CYP}}{\text{RI}_{CYP} \cdot \text{TDI}_{CYP}} + \text{CL}_{int,bile} \right)}{\left( \sum \frac{\text{CL}_{int,CYP}}{\text{RI}_{CYP} \cdot \text{TDI}_{CYP}} + \text{CL}_{int,bile} \right)^{-1}}
\]

(5)

\( \text{RI}_{OATP} \) is the competitive inhibition term (Eq. 6) for active hepatic uptake (International Transporter et al., 2010; USFDA, 2012; Barton et al., 2013). \( \text{RI}_{CYP}, \text{TDI}_{CYP} \), and \( \text{RI}_{OAT} \) defined above.

\[
\begin{align*}
\text{RI}_{OATP} &= 1 + \sum \frac{[I_{u,max,in}]}{K_{i,OATP}}; \quad \text{RI}_{CYP} = 1 + \sum \frac{[I_{u,max,in}]}{K_{i,CYP}}; \quad \text{RI}_{OAT3} = 1 + \sum \frac{[I_{u,max}]}{K_{i,OAT3}} \\
\text{TDI}_{CYP} &= \frac{k_{deg,h} + \frac{[I_{u,max,in}]}{[I_{u,max,in} + K_i]} \cdot k_{inact}}{k_{deg,h}}
\end{align*}
\]

(6)

(7)

\( K_i \) (\( K_{i,OATP}, K_{i,CYP} \) and \( K_{i,OAT3} \)) is the inhibition constant, \( I_{u,max} \) is the maximum unbound plasma perpetrator concentration and \( I_{u,max,in} \) is the maximum unbound perpetrator concentration at the inlet to liver, calculated using Eq. 8. \( k_{inact} \) and \( K_i \) are the maximal inactivation rate constant and
the inhibitor concentration that supports half the maximal rate of inactivation, respectively. $k_{\text{deg},h}$ is the apparent first-order degradation rate constant of the affected hepatic enzyme. The following interaction parameters were used: gemfibrozil $K_{\text{IATP}}$ (2.54µM), Gem-Glu $K_{\text{IATP}}$ (7.9µM), gemfibrozil $K_{\text{CYP2C8}}$ corrected for binding to microsomal protein (6.9µM), Gem-Glu KI corrected for binding to microsomal protein and $k_{\text{inact}}$ against CYP2C8 (7.9µM and 12.6h$^{-1}$), gemfibrozil $K_{\text{OAT3}}$ (3.4µM), Gem-Glu $K_{\text{OAT}}$ (9.9µM), itraconazole and 4-hydroxy itraconazole $K_{\text{CYP3A}}$ (0.0013µM and 0.014µM, respectively) (Schneck et al., 2004; Nakagomi-Hagihara et al., 2007a; Nakagomi-Hagihara et al., 2007b; VandenBrink et al., 2011; Barton et al., 2013).

Due to minor effect on CYP3A and intestinal transporters, gemfibrozil is assumed to have no effect on $F_a.F_g$, thus the change in intestinal absorption and extraction is minimal ($F_a`.F_g`/F_a.F_g \sim 1.0$). However, the change in $F_g$ due to CYP3A inhibition (as described elsewhere (Fahmi et al., 2008)) by itraconazole was considered when predicting DDIs involving the combination of gemfibrozil and itraconazole. $I_{u,\text{gut}}$, the free intestinal concentration of the perpetrator, was estimated by Eq. 9.

$$[I_{u,max,in}] = f_{u,b} \left( [I_{max,b}] + \frac{\text{Dose}.K_a.F_a.F_g}{Q_h} \right)$$

$$[I_{u,\text{gut}}] = \frac{\text{Dose}.K_a.F_a.f_{u,\text{gut}}}{Q_{\text{gut}}}$$

Dose, $I_{max,b}$, $K_a$, $f_{u,\text{gut}}$ and $Q_{\text{gut}}$ (248 mL/min (Fahmi et al., 2008)) represent total dose given orally, maximum total blood concentration, absorption rate constant, fraction unbound in the gut and enterocytic blood flow, respectively. Consistent with the earlier reports, $k_{\text{deg},h}$ was assumed to be 0.019h$^{-1}$ for hepatic CYP2C8 (Fahmi et al., 2008; Lai et al., 2009).
Changes in active renal secretion (CL\textsubscript{sec}) caused by inhibition of OAT3 by gemfibrozil is described by a basic model (Feng et al., 2013).

\[
\text{CL\textsubscript{sec}'} = \frac{\text{CL\textsubscript{sec}}}{1 + \frac{I\text{max, u}}{K_I\text{OAT3}}}
\]  

(10)

\textit{In vivo} \text{CL\textsubscript{int,h}} was calculated using the well-stirred liver model (Pang and Rowland, 1977).

\[
\text{CL\textsubscript{int,h}} = \frac{\text{CL\textsubscript{h}}}{f_u,h \cdot \left(1 - \frac{\text{CL\textsubscript{h}}}{Q_h}\right)}
\]  

(11)

where \text{CL\textsubscript{h}} [= (\text{CL\textsubscript{p}} - \text{CL\textsubscript{r}})/R_b] is the hepatic blood clearance obtained from intravenous total plasma clearance corrected for renal clearance and blood-to-plasma ratio (R\textsubscript{b}).

**PBPK Modeling and Simulations**

Modeling and simulations were performed using the population-based ADME simulator, Simcyp (version 13, Simcyp Ltd, Sheffield, UK). Each simulation was performed for 50 virtual populations of healthy subjects (5 trials × 10 subjects). PBPK models for gemfibrozil and Gem-Glu were similar to that described elsewhere (Varma et al., 2012; Barton et al., 2013). Briefly, a model for gemfibrozil was constructed using clinical first-order absorption rate (F\text{a}) observed volume of distribution (0.08 L/kg) and oral clearance (6.0 L/h) (Table 1). Based on the \textit{in vitro} data, the model considered 79% of gemfibrozil dose underwent glucuronidation to form Gem-Glu, while the rest was accounted for by CYP3A-dependent metabolism (Kilford et al., 2009). A lag-time of 15min 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
gemfibrozil, fu and blood/plasma ratio. Due to the lack of disposition data for the metabolite, the Vss (0.1 L/kg) and biliary intrinsic clearance (5.2 µL/min/10^6 cells) 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: gemfibrozil KiOATP (2.54µM), Gem-Glu KiOATP (7.9µM), gemfibrozil KiCYP2C8 corrected for binding to microsomal protein (6.9µM), Gem-Glu KI corrected for binding to microsomal protein and k inact against CYP2C8 (7.9µM and 12.6h⁻¹), gemfibrozil KiCYP3A (68.1µM) and Gem-Glu KiCYP3A (103.7µM) (0.0013µM and 0.014µM, respectively) (Schneck et al., 2004; Nakagomi-Hagihara et al., 2007b; VandenBrink et al., 2011; Barton et al., 2013). Inhibition of renal transporters was not included in the PBPK model as the victim drugs evaluated with PBPK modeling are not eliminated in the urine.

For pioglitazone, a model was developed assuming rapid-equilibrium between liver and blood compartments using the physicochemical properties, in vitro preclinical data such as human plasma fu, blood-to-plasma ratio, metabolic intrinsic clearance values, etc. (Table 1). In the case of rosiglitazone, the model (also assumed rapid-equilibrium) was adopted from Simcyp V13 compound files. Models for 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 Rodger et al. method (Rodgers and Rowland, 2006) considering rapid equilibrium between blood and tissues was adopted to obtain the distribution into all organs, except 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 concentration-time profile, while fixing the rest of the parameters (Varma et al., 2012; Barton et al., 2013). Absorption phase in the model was captured by the ADAM model using Caco-2 permeability data.
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; Varma et al., 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 3 false positive (predicted >1.25-fold, observed ≤1.25-fold) and 3 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 positive predictions, although the predicted AUCRs are below 1.5. The 3 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), while 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 following the first dose of 600mg gemfibrozil. Moreover, consideration of Gem-Glu increased the inhibition of OATP1B1 (Fig. 2C).

PBPK models for 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 OATP1B1 and CYP2C8/3A4 dual substrates, repaglinide and cerivastatin. Models were constructed using the *in silico*, *in vitro* and the intravenous pharmacokinetic data and further verified using the oral pharmacokinetic profiles (Fig. 3). Model predictions of pioglitazone-gemfibrozil interaction are similar to that observed in the clinic when considering TDI by Gem-Glu, without which no significant AUC change was predicted (Fig. 3A). Similarly, in case of rosiglitazone interactions, parent alone predicted no AUC change (<1.25-fold) while 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. 3C and 3D). 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 following 600mg gemfibrozil is enough to move the DDI category from weak or no interaction (<2 fold) to a moderate risk category (2-5 fold) (Fig. 4). Change in pioglitazone and rosiglitazone AUC was minimal above metabolite-to-parent ratio of 0.1, while significant increase in 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 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, (i) implement the learnings for the prospective predictions of gemfibrozil interactions early in the drug development and strategize the clinical studies, (ii) understand the quantitative role of a major circulating metabolite in governing the magnitude of the DDI, and (iii) 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 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 under-prediction 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 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 SCHH, and developed mechanistic models incorporating transporter-enzyme interplay. In terms of extended clearance concept (Shitara et al., 2006; Li et al., 2014), total hepatic uptake for repaglinide and cerivastatin is significantly higher than passive uptake and the passive uptake and metabolic intrinsic clearance are within a similar range (Table 1). Therefore, the systemic clearance of these drugs decrease by inhibiting active uptake or metabolism, with a much larger impact caused by simultaneous inhibition of both processes. Earlier studies suggested in vitro-in vivo disconnect in the repaglinide fm_{CYP2C8} on the basis of a collection of clinical repaglinide-gemfibrozil DDI reports (Bidstrup et al., 2003; Kajosaari et al., 2005a; Honkalammi et al., 2011a; Honkalammi et al., 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 fm_{CYP2C8} 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, cerivastatin-gemfibrozil interaction of ~5-fold can be recovered with in vitro fm_{CYP2C8} 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 larger than expected clinical interaction with gemfibrozil is due to simultaneous inhibition of both OATP1B1-mediated active uptake and CYP2C8-based metabolism.

Recent regulatory guidances suggest use of repaglinide as a clinical probe for the assessment of CYP2C8 and OATP1B1 inhibition (EMA, 2012; USFDA, 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 victim drug. Due to the lack of alternative sensitive probes, CYP2C8 substrates with no active hepatic uptake and 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 is to determine the quantitative contribution of the major metabolite(s) to the *in vivo* DDIs, and examine the application of regulators recommendation to trigger *in vitro* investigation of interaction potential of metabolites when present at ≥25% of parent AUC. Sensitivity analysis on the impact of metabolite-to-parent ratio on AUCR suggested that Gem-Glu exposure at ~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 following a 600mg 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. 4C and 4D). These model predictions are consistent with clinical findings where sub-therapeutic gemfibrozil dose (30mg; 20 times lower than therapeutic dose) caused ~3.4-fold increase in repaglinide AUC while further increase in AUCR was noted with higher doses (100mg and 600mg gemfibrozil doses yielded repaglinide AUCR of ~5.5 and ~7, respectively) (Honkalammi et al., 2012). Overall, the default metabolite-
to-parent exposure cut-off ($\geq 25\%$) may not firmly reflect upon 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 (EMA, 2012; USFDA, 2012). However, due to its multiple interaction mechanisms, the interpretation of the clinical study data will be challenging for investigational drugs that serves as OATP1B1 substrates also. Our predictions suggest use of subtherapeutic doses of gemfibrozil (100mg b.i.d.) in such situations, where the competitive inhibition of transporters is insignificant but the CYP2C8 inactivation is almost complete (Fig. 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 OATP *in vitro*; but no DDI with statins is evident (Patino-Rodriguez et al., 2014). Beyond Gem-Glu, only clopidogrel-acyl-β-D-glucuronide showed 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 ~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 renders CYP2C8 inactivation – which could flag probable glucuronides for early in vitro DDI assessment.

This study reinforces the utility of in vitro data and the modelling 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 difference 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. While early predictions with consideration to sensitivity analysis of uncertain parameters, particularly for metabolite exposure, could be helpful at the drug discover stage, there is need to refine the models of both parent and metabolite(s) as the clinical pharmacokinetic and human ADME data become available. Mechanistic static models described here will be valuable in the early discovery and development stage, while 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 co-administration, and potentially avoid unnecessary clinical studies.
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Authorship contributions

Participated in research design: M.V.S.V., J.L., Y.B., E.K., A.D.R.

Performed data analysis: M.V.S.V., J.L.

Wrote or contributed to the writing of the manuscript: M.V.S.V., J.L., Y.B., E.K., A.D.R.
REFERENCES


LEGENDS FOR FIGURES

Fig. 1. Prediction of gemfibrozil drug interactions based on static net-effect models. Observed versus predict AUCRs when considering the interaction potential of parent alone (A) and when assuming the combined effect of both parent and Gem-Glu (B). Static predictions are based on the net-effect model for victim drugs that are not substrates to active hepatic uptake (circles) and using an extended net-effect model for victim drugs that are OATP1B1 substrates (triangles). Open data points represent interactions with gemfibrozil alone treatment. Filled data points represent interactions with gemfibrozil and itraconazole combination treatment. Diagonal solid, dotted and dashed lines indicate unity, 1.5-fold and 2-fold range, respectively. Vertical and horizontal dotted lines drawn at AUCR of 1.25 represent bioequivalence boundaries.

Fig. 2. Pharmacokinetics and CYP2C8/OATP1B1 interaction profiles of gemfibrozil. PBPK model prediction of the plasma concentration-time profiles of gemfibrozil (A) and Gem-Glu (B) following 600mg b.i.d. gemfibrozil oral dosing. Data points are mean observed values from separate studies (Tornio et al., 2008; Honkalammi et al., 2011b). (C) Predicted in vivo CYP2C8 inhibition potential (red curves) and OATP1B1 inhibition potential (green curves) of parent alone (dashed lines) and parent/metabolite pair (solid lines) following 600mg b.i.d. gemfibrozil. (D) CYP2C8 inhibition potential (red curves) and OATP1B1 inhibition potential (green curves) of parent/metabolite pair following 30mg b.i.d. (dashed lines), 100mg b.i.d. (dashed-dotted lines) and 600mg b.i.d. (solid lines) gemfibrozil.
Fig. 3. PBPK prediction of pharmacokinetic interactions of CYP2C8 substrates (pioglitazone (A) and rosiglitazone (B)) and OATP1B1-CYP2C8 dual substrates (cerivastatin (C) and repaglinide (D)) following 600mg b.i.d. gemfibrozil. Predicted mean plasma concentration-time profiles in control (dashed curves) and gemfibrozil treatment when considering interaction potential of parent alone (dotted curves) or assuming the combined effect of both parent and Gem-Glu (solid curves), are represented. Open and filled data points are mean observed plasma concentrations in the control and gemfibrozil treatment subjects (Niemi et al., 2003a; Niemi et al., 2003b; Deng et al., 2005; Jaakkola et al., 2005; Honkalammi et al., 2011a; Honkalammi et al., 2012; Aquilante et al., 2013) – where available, normalized data from multiple reports are shown.

Fig. 4. PBPK model based prediction of the effect of metabolite-to-parent ratio on the exposure increase of victim drugs pioglitazone (A), rosiglitazone (B), cerivastatin (C) and repaglinide (D). Data points are mean observed AUCR (filled) and Cmax ratio (open) from separate studies when available. Vertical dotted lines represent 25% metabolite-to-parent exposure cut-off, above which, in vitro investigation of metabolites interaction potential is recommended. Horizontal dotted lines represent DDI categories (<1.25, no interaction; 1.25-2, weak interaction; 2-5, modest interaction; >5, strong interaction).
Table 1. Summary of victim drug input parameters for PBPK modeling and simulations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pioglitazone</th>
<th>Rosiglitazone</th>
<th>Repaglinide</th>
<th>Cerivastatin</th>
<th>Gemfibrozil</th>
<th>Gemfibrozol-1-O-β-glucuronide</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
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<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>
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<td>Ampholyte</td>
<td>Monoprotic acid</td>
<td>Monoprotic acid</td>
<td>Monoprotic acid</td>
</tr>
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<td>pKₐ</td>
<td>5.53</td>
<td>6.25 &amp; 6.32</td>
<td>4.19 &amp; 5.78</td>
<td>5</td>
<td>4.75</td>
<td>2.68</td>
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<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>
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<td>Blood/plasma ratio</td>
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<td>0.57</td>
<td>0.62</td>
<td>0.76</td>
<td>0.825</td>
<td>0.825</td>
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<td></td>
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<td></td>
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<tr>
<td>Absorption type</td>
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<td>1st order</td>
<td>ADAM</td>
<td>ADAM</td>
<td>1st order</td>
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<tr>
<td>Fraction absorbed</td>
<td>0.98</td>
<td>1 (Ka=3.6h⁻¹)</td>
<td>0.99</td>
<td>0.75</td>
<td>1 (Ka=3h⁻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>
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<td>Absorption Scalar</td>
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<td>1.873</td>
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<tr>
<td>fᵤₑ₉ₑ₉ₑ</td>
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<td>1</td>
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<td><strong>Distribution</strong></td>
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<tr>
<td>Distribution model</td>
<td>Minimal PBPK</td>
<td>Minimal PBPK</td>
<td>Full PBPK</td>
<td>Full PBPK</td>
<td>Minimal PBPK</td>
<td>Minimal PBPK</td>
</tr>
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<td>Vss (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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<td>CLₑ₉ₑ₉ₑ,CYP2C9 (µL/min/mg)</td>
<td>27.5 (HLM)</td>
<td>191 (HLM)</td>
<td>93 (HLM)</td>
<td>19.1 (HLM)</td>
<td></td>
<td></td>
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<tr>
<td>CLₑ₉ₑ₉ₑ,CYP3A4 (µL/min/mg)</td>
<td>1.5 (HLM)</td>
<td>38 (HLM)</td>
<td>12.7 (HLM)</td>
<td>0.29 (rCYP)²</td>
<td></td>
<td></td>
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<tr>
<td>CLₑ₉ₑ₉ₑ,others (µL/min/mg)</td>
<td>6.1 (CYP2C19-HLM)</td>
<td>102 (CYP2C9-HLM)</td>
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<td></td>
<td>65 (UGT2B7-HLM)</td>
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<tr>
<td>Biliary CLₑ₉ₑ₉ₑ (µL/min/10⁻⁶cells)</td>
<td></td>
<td></td>
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<td>5.2</td>
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<tr>
<td>Renal clearance (L/h)</td>
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## Hepatobiliary transport

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<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
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<tr>
<td>Liver unbound fraction</td>
<td>0.143/0.028</td>
<td>0.97/0.025</td>
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<td>(Intra-/extra-cellular)</td>
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<td></td>
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<tr>
<td>Passive diffusion (µL/min/10⁶ cells)</td>
<td>22</td>
<td>17.5</td>
</tr>
<tr>
<td>CL_{int,active} (µL/min/10⁶ cells)</td>
<td>35.5</td>
<td>16.8</td>
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<td>Scaling factor (Active uptake)</td>
<td>18.7.tbl</td>
<td>30.5.tbl</td>
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### References

- [Jaakkola et al., 2006](#)
- [Barton et al., 2013](#)
- [Muck et al., 1997; Varma et al., 2014](#)
- [Barton et al., 2013](#)
- [Barton et al., 2013](#)

*Estimated by fitting to intravenous pharmacokinetics data. See Methods.*

†Estimated by fitting to intravenous data. See Methods.

ADAM, Advanced dissolution, absorption and metabolism model; P, partition coefficient; pKa, acid dissociation constant.

†Rosiglitazone model was adopted from Simcyp V13 compound files.

‡Models developed in this current study based on literature in vitro data (pioglitazone) or inhouse in vitro data (cerivastatin).

§Model adopted from a previous report (Barton et al., 2013).

¶Model modified from a previous report (Barton et al., 2013).

ΩCL_{int,CYP3A4} in µL/min/pmol.
Table 2. Summary of victim-gemfibrozil DDI predictions using mechanistic static models.

<table>
<thead>
<tr>
<th>Static model</th>
<th>Victim drug</th>
<th>Gemfibrozil dose</th>
<th>$f_{\text{m}_{\text{CYP2C8}}}$</th>
<th>$F_{\text{Renal}}$</th>
<th>$F_{\text{sec}}$</th>
<th>Observed AUCR</th>
<th>Predicted (gemfibrozil only)</th>
<th>Predicted (gemfibrozil &amp; Gem-Glu)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>(S)-warfarin</td>
<td>600 mg (8 days)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Kaminsky and Zhang, 1997; Lilja et al., 2005)</td>
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<tr>
<td>NE</td>
<td>lovastatin</td>
<td>600 mg (3 days)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Wang et al., 1991; Chen et al., 2012)</td>
<td></td>
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<tr>
<td>NE</td>
<td>imatinib</td>
<td>600 mg (6 days)</td>
<td>0.25</td>
<td>0.05</td>
<td>0.46</td>
<td>0.9</td>
<td>1.1</td>
<td>1.3</td>
<td>(Nebot et al., 2010; Filppula et al., 2013)</td>
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<tr>
<td>NE</td>
<td>(R)-warfarin</td>
<td>600 mg (8 days)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Kaminsky and Zhang, 1997; Lilja et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>brivaracetam</td>
<td>600 mg (7 days)</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Nicolas et al., 2012)</td>
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<tr>
<td>NE</td>
<td>lovastatin</td>
<td>600 mg (3 days)</td>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Wang et al., 1991; Kyrklund et al., 2001)</td>
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<td>NE</td>
<td>zopiclone</td>
<td>600 mg (3 days)</td>
<td>0.3</td>
<td>0.03</td>
<td>0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4</td>
<td>(Becquemont et al., 1999; Tornio et al., 2006)</td>
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<td>zafirlukast</td>
<td>600 mg (5 days)</td>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>(Dekhuijzen and Koopmans, 2002; Karonen et al., 2011)</td>
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<tr>
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<td>600 mg (3 days)</td>
<td>0.23</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
<td>(Tornio et al., 2007; Chang et al., 2008)</td>
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<tr>
<td>NE</td>
<td>alogliptin</td>
<td>600 mg (7 days)</td>
<td>0</td>
<td>0.71</td>
<td>0.38</td>
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<td>1.2</td>
<td>(Niemi et al., 2001)</td>
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<td>600 mg (2.5 days)</td>
<td>0.2</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>(Tornio et al., 2007; Chang et al., 2008)</td>
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<tr>
<td>NE</td>
<td>(R)-ibuprofen</td>
<td>600 mg (3 days)</td>
<td>0.25</td>
<td>1.3</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
<td>(Tornio et al., 2007; Chang et al., 2008)</td>
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<tr>
<td>NE</td>
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<td>600 mg (3 days)</td>
<td>0</td>
<td>1.4</td>
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<td>1.0</td>
<td>(Backman et al., 2000; Prueksaritanont et al., 2003)</td>
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<td>atorvastatin acid</td>
<td>600 mg (7 days)</td>
<td>0</td>
<td>1.4</td>
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<td>(Whitfield et al., 2011)</td>
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<td>ezetimibe</td>
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<td>1.0</td>
<td>(Ghosal et al., 2004; Reyderman et al., 2004; Kosoglou et al., 2005)</td>
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*This article has not been copyedited and formatted. The final version may differ from this version.*
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NE - net effect model; ENE – extended net-effect model; Itra –itraconazole *value in the parenthesis indicate fmCYP3A4.
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.