Dose-Dependent Interaction between Gemfibrozil and Repaglinide in Humans: Strong Inhibition of CYP2C8 with Subtherapeutic Gemfibrozil Doses

Johanna Honkalammi, Mikko Niemi, Pertti J. Neuvonen and Janne T. Backman

Department of Clinical Pharmacology, University of Helsinki

and HUSLAB, Helsinki University Central Hospital, Helsinki, Finland
Running title:

DOSE-DEPENDENT INHIBITION OF CYP2C8 BY GEMFIBROZIL

Address correspondence to:

Janne T. Backman, MD
PO Box 705, FI-00029 HUS, Finland
Tel: +358 9 471 73914
Fax: +358 9 471 74039
E-mail: janne.backman@helsinki.fi

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ABBREVIATIONS: AUC, area under the concentration-time curve; C_{hu}/C_{p,tot}, the hepatocyte (unbound) to plasma (total) concentration ratio; C_{max}, peak concentration; f_{m,CYP2C8}, the fraction of repaglinide dose metabolised by CYP2C8; f_{t,OATP1B1}, fraction of dose transported by OATP1B1; k_e, the first-order degradation rate constant; K_i, the inhibitor concentration that supports half the maximal rate of enzyme inactivation; k_{inact}, maximal rate of inactivation; OATP,
organic anion transporting polypeptide; P450, Cytochrome P450; \textit{SLCO1B1}, solute carrier organic anion transporter family, member 1B1 gene encoding for OATP1B1; SNP, single nucleotide polymorphism; $t_{\text{max}}$, time to peak concentration.
Abstract

Gemfibrozil 1-O-β-glucuronide inactivates cytochrome P450 (P450) 2C8 irreversibly. We investigated the effect of gemfibrozil dose on CYP2C8-activity in humans using repaglinide as a probe drug. In a randomized 5-phase cross-over study, ten healthy volunteers ingested 0.25 mg repaglinide 1 hour after different doses of gemfibrozil or placebo. Concentrations of plasma repaglinide, gemfibrozil, their metabolites and blood glucose were measured. A single gemfibrozil dose of 30, 100, 300 and 900 mg increased the area under the concentration-time curve (AUC₀⁻∞) of repaglinide 1.8-, 4.5-, 6.7- and 8.3-fold (P < 0.001), and its peak concentration 1.4-, 1.7-, 2.1- and 2.4-fold (P < 0.05), compared to placebo, respectively. Gemfibrozil pharmacokinetics was characterized by a slightly more than dose-proportional increase in the AUC of gemfibrozil and its glucuronide. The gemfibrozil-repaglinide interaction could be mainly explained by gemfibrozil 1-O-β-glucuronide concentration-dependent mechanism-based inhibition of CYP2C8, with a minor contribution by competitive inhibition of organic anion transporting polypeptide (OATP) 1B1 at the highest gemfibrozil dose. The findings are consistent with ~50% inhibition of CYP2C8 already with a single 30 mg dose of gemfibrozil, and >95% inhibition with 900 mg. In clinical drug-drug interaction studies, a single 900 mg dose of gemfibrozil can be used to achieve nearly complete inactivation of CYP2C8.
Introduction

Cytochrome P450 (P450) 2C8 is one of the major drug-metabolising P450 forms, and it accounts for approximately 6% of the hepatic P450 content (Totah and Rettie, 2005; Lai et al., 2009). CYP2C8 can be inhibited in vitro by many commonly used drugs, e.g., gemfibrozil, montelukast, isoniazid, nortriptyline, amiodarone, verapamil and trimethoprim (Polasek et al., 2004; Walsky et al., 2005a; Walsky et al., 2005b; Lai et al., 2009). The importance of CYP2C8-mediated drug-interactions is increasing continuously, as the list of CYP2C8 substrates and therefore, the list of potential victim drugs of CYP2C8-mediated interactions, is increasing. To date, e.g., paclitaxel, cerivastatin, loperamide, rosiglitazone, repaglinide, amiodarone, amodiaquine and montelukast have been recognized as CYP2C8 substrates (Rahman et al., 1994; Ohyama et al., 2000; Backman et al., 2002; Wang et al., 2002; Niemi et al., 2003a; Kim et al., 2004; Jaakkola et al., 2005; Kajosaari et al., 2005a; Totah and Rettie, 2005; Niemi et al., 2006; Lai et al., 2009; Karonen et al., 2010; Filppula et al., 2011).

When developing new therapeutic agents, it is important, among other things, to assess whether their metabolism is dependent on CYP2C8 (Huang et al., 2007; Huang et al., 2008).

Repaglinide is a short-acting meglitinide class antidiabetic drug, which has been recommended as a probe substrate for studying CYP2C8 activity (FDA, 2006). The formations of the repaglinide main metabolites M2 and M4 are mainly mediated by CYP2C8, whereas M1 is mainly formed by CYP3A4 (Bidstrup et al., 2003; Kajosaari et al., 2005a; Kajosaari et al., 2005b). The changes in pharmacokinetic variables of parent repaglinide and its metabolites reflect changes in CYP2C8 activity (Niemi et al., 2003a; Niemi et al., 2003b; Niemi et al., 2004; Backman et al., 2009; Honkalammi et al., 2011). In addition, genetic variability in the
SLCO1B1 gene encoding the hepatic uptake transporter OATP1B1 transporting repaglinide from blood to the hepatocytes (Kalliokoski and Niemi, 2009) can affect the pharmacokinetics of repaglinide (Niemi et al., 2005).

Gemfibrozil, a fibrate class antihyperlipidaemic agent, is the strongest known inhibitor of CYP2C8 in vivo (Backman et al., 2002). Therefore, it has been recommended as a model inhibitor of CYP2C8 for in vivo studies by the Food and Drug Administration and European Medicines Agency. Among clinically significant inhibitors of P450 enzymes gemfibrozil is unique, since its inhibitory effect is based on mechanism-based inhibition of CYP2C8 by its “phase 2” metabolite, gemfibrozil 1-O-β-glucuronide (Wang et al., 2002; Shitara et al., 2004; Ogilvie et al., 2006; Baer et al., 2009).

In clinical use, gemfibrozil is administered usually as two 600 mg doses daily and in some countries as a single 900 mg daily dose. In our previous studies, administration of 600 mg gemfibrozil twice daily has increased the area under the plasma concentration time curve (AUC) of repaglinide about 8-fold (Niemi et al., 2003a), that of cerivastatin 5-6-fold (Backman et al., 2002) and that of montelukast 4-5-fold (Karonen et al., 2010), which indicates strong inhibition of the CYP2C8 enzyme. The CYP2C8-inhibitory effect of gemfibrozil can have serious clinical consequences, as demonstrated by the gemfibrozil-cerivastatin interaction: roughly one third of the fatal cases of cerivastatin-induced rhabdomyolysis involved coadministration of gemfibrozil (Backman et al., 2002). In addition, gemfibrozil and its glucuronide may competitively inhibit OATP1B1 and therefore interfere with repaglinide pharmacokinetics (Shitara et al., 2004). Recent studies using repaglinide as a CYP2C8 probe substrate have shown that the inactivation of CYP2C8 occurs rapidly in humans (Honkalammi et al., 2011), and that the recovery of CYP2C8 takes
place slowly after cessation of gemfibrozil administration (Tornio et al., 2008; Backman et al., 2009). These findings are consistent with the mechanism-based nature of the inhibitory effect. However, the dependence of CYP2C8 inactivation on the dose of gemfibrozil is not known. This information would be particularly relevant for the selection of gemfibrozil dose when it is used as a CYP2C8 probe inhibitor.

The aim of the present study was to investigate the dose-dependency of the inactivation of CYP2C8 by gemfibrozil using repaglinide as a CYP2C8-probe drug, and to apply enzyme and transporter inhibition models to the obtained in vivo data, in order to better understand the concentration-dependency and mechanism of the observed interaction. We used a study design, where 10 healthy volunteers were given a single dose of repaglinide after an oral dose of gemfibrozil 30, 100, 300 or 900 mg or placebo in a double-blind crossover study of 5 phases.
Methods

Subjects. Ten healthy volunteers (1 female and 9 males, aged 20-26 years, body mass index 21-27 kg/m²) participated in the study after giving written informed consent (Table 1), and their health was ascertained by medical history, physical examination, and routine laboratory tests before entering the study. None of the volunteers were smokers or used any continuous medication. The sample size was estimated to be sufficient to detect a 30% change in the AUC₀-∞ of repaglinide with a power of 80% (alpha-level 5%).

Study design. The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District and by the National Agency for Medicines. A randomized crossover study with 5 phases and a washout period of 2 weeks between phases was carried out. All subjects completed all 5 phases of the study. On the study day, a single oral dose of 0.25 mg repaglinide (half of a 0.5 mg tablet of NovoNorm; Novo Nordisk, Bagsværd, Denmark) was administered with 150 ml water at 9 AM after an overnight fast and 1 hour after a single 30, 100, 300 or 900 mg dose of gemfibrozil or placebo. Gemfibrozil and placebo capsules were prepared and analysed using methods described in the European Pharmacopoeia (Ph. Eur.) by the Helsinki University Central Hospital Pharmacy. Placebo capsules contained microcrystallised cellulose (Cellulos. Microcryst., Orion Pharma), and gemfibrozil capsules contained pulverised gemfibrozil (prepared from Lopid 600 mg tablets; Gödecke, Freiburg, Germany) and microcrystallised cellulose, as appropriate. The gemfibrozil content of the capsules was measured using the liquid chromatography–tandem mass spectrometry system described below.
Food intake was identical in all phases: a standardized light breakfast 15 minutes after repaglinide administration, snacks after 1 and 2 hours, a warm meal after 3 hours, and snacks after 7 and 9 hours. Additional carbohydrates, glucose solution for intravenous use and glucagon for intramuscular use were available for use in case of severe hypoglycaemia.

**Sampling.** Timed blood samples (4 or 9 ml each) were drawn from a cannulated forearm vein 60, 30 and 5 min before and at 15, 30, 45, 60, 80 and 100 minutes, and 2, 2.5, 3, 4, 5, 7 and 9 hours after the administration of repaglinide. Blood samples were taken into EDTA containing tubes. Blood glucose concentrations were measured immediately after sampling using a Precision Exceed device (Abbott Diabetes Care Ltd, Witney Oxon, UK). Plasma was separated within 30 minutes and stored at -70°C until analysis.

**Determination of drug concentrations.** Concentrations of repaglinide and its metabolites M1, M2, and M4 were measured in plasma samples by use of an API 3000 liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada), as described earlier (Tornio et al., 2008; Backman et al., 2009). The limit of quantification for repaglinide was 0.01 ng/ml, and interday coefficients of variation (CV) were 4.6% at 0.1 ng/ml, 2.6% at 2.0 ng/ml, and 2.3% at 20 ng/ml (n=6). The limit of quantification for repaglinide M1 and M2 was 0.05 ng/ml, and interday CVs were 14.7% and 8.9% at 0.1 ng/ml and 7.5% and 11.5% at 2.0 ng/ml for M1 and M2, respectively (n=6). Because an authentic metabolite standard for M4 was not available, M4 concentrations are given in arbitrary units (units per milliliter) relative to the ratio of the peak height of M4 to that of the internal
standard in the chromatogram. The limit of quantification for M4 was based on a
signal-to-noise ratio of more than 10:1. The plasma concentrations of gemfibrozil and
gemfibrozil 1-O-β-glucuronide were determined by use of API 2000 liquid
chromatography–tandem mass spectrometry system (Sciex Division of MDS)
(Backman et al., 2009; Honkalammi et al., 2011). Gemfibrozil-d6 and gemfibrozil 1-
O-β-glucuronide-d6 served as internal standards. The limits of quantification for
gemfibrozil and gemfibrozil 1-O-β-glucuronide were 2.5 ng/ml, and interday CVs
were 4.4-8.7% and 3.0-7.0% at relevant plasma concentrations, respectively.

Pharmacokinetics. The pharmacokinetics of repaglinide and its metabolites M1, M2,
and M4 were characterized by $C_{\text{max}}$, time to $C_{\text{max}}$ ($T_{\text{max}}$), areas under the plasma
concentration-time curve (AUC$_{0-9\ h}$ and AUC$_{0-\infty}$; AUC$_{0-3\ h}$ for M4) and elimination
half-life ($t_{\frac{1}{2}}$), calculated by noncompartmental analysis using MK-Model, version 5.0
(Biosoft, Cambridge, UK). The terminal log-linear part of each concentration-time
curve was identified visually. The elimination rate constant (kel) was determined by
linear regression analysis of the log-linear part of the plasma concentration-time
curve. The $t_{\frac{1}{2}}$ was calculated by the equation $t_{\frac{1}{2}} = \ln2/\text{kel}$. The AUC values were
calculated by use of the linear trapezoidal rule for the rising phase of the plasma
concentration-time curve and the log-linear trapezoidal rule for the descending phase,
with extrapolation to infinity, when appropriate, by dividing the last measured
concentration by kel. The pharmacokinetics of gemfibrozil and gemfibrozil 1-O-β-
glucuronide were characterized by concentration at 1 h postdose ($C_{1\ h}$), $C_{\text{max}}$, $T_{\text{max}}$, $t_{\frac{1}{2}}$
and AUC.
Pharmacodynamics. The pharmacodynamics of repaglinide were characterized by baseline blood glucose concentration, minimum blood glucose concentration and mean blood glucose concentration during the study day, from 0 to 9 hours after repaglinide intake.

Genotyping. For genotyping, a 12-ml EDTA blood sample was drawn from each subject and stored at -20°C. Genomic deoxyribonucleic acid (DNA) was extracted with standard methods (Qiaamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). The subjects were genotyped for the $CYP2C8^{*3}$ (c.416G>A and c.1196A>G) and $CYP2C8^{*4}$ (c.792C>G) alleles and the $SLCO1B1$ c.388A>G and c.521T>C single nucleotide polymorphisms, defining the $SLCO1B1^{*1B}$ (GT), $^{*5}$ (AC), and $^{*15}$ (GC) haplotypes (Kalliokoski and Niemi, 2009), with TaqMan® genotyping assays on an Applied Biosystems 7300 Real-Time PCR system (Pasanen et al., 2006).

Statistical analysis. The results are expressed as mean values ± SD in the text, tables and figures, unless otherwise indicated. The pharmacokinetic and pharmacodynamic variables between the study phases were compared by the paired t-test. To avoid false negative conclusions and because the direction of the interaction has been documented previously, no Bonferroni correction for multiple comparisons was applied, and differences were considered statistically significant at $P < 0.05$. The $T_{max}$ data were compared using the Wilcoxon signed rank test.

The dose-proportionality of gemfibrozil pharmacokinetics was estimated by regression analysis with the power model approach using a logarithmically transformed from of the equation $\text{AUC}_{0-\infty} = e^\alpha \cdot \text{dose}^\beta$ after logarithmic transformation.
of the AUC data, where statistically significant deviation of the term $\beta$ from unity indicates non-linearity.

To characterize the dose-dependency of the gemfibrozil-repaglinide interaction, we applied several static enzyme and transporter inhibition models to the relationship between the plasma concentrations of gemfibrozil or its 1-O-$\beta$-glucuronide and the increase in the AUC of repaglinide, with the following assumptions and simplifications:

1) The increment in repaglinide AUC was due to a single mechanism only, i.e., either irreversible mechanism-based inactivation of hepatic CYP2C8 or competitive inhibition of OATP1B1 by gemfibrozil 1-O-$\beta$-glucuronide, or competitive inhibition of hepatic CYP2C8 or OATP1B1 by gemfibrozil, and no other kind of changes in the activity of relevant enzymes or transporters was involved.

2) For mechanism-based inhibition, the conditions were assumed to approximate static “steady-state” conditions, where the average (or peak) plasma concentration of gemfibrozil 1-O-$\beta$-glucuronide during the study day (0-10 hour after gemfibrozil intake) reflects its steady-state concentration in hepatocytes and repaglinide AUC reflects the average CYP2C8 activity in hepatocytes.

3) For all models, it was assumed that all possible parallel metabolism or transport/elimination pathways can be described as first-order processes and that the gemfibrozil treatment has no effect on such parallel processes other than CYP2C8 and OATP1B1.

For mechanism-based inhibition, the fold increase in repaglinide AUC caused by the different gemfibrozil doses in the ten subjects was expressed using the equation: $\frac{AUC_i}{AUC_c}=1/(f_{in,CYP2C8}((k_{inact}/K_i)\cdot([I]/[c]))+1-f_{in,CYP2C8})$, where $k_{inact}$ is the maximal rate of CYP2C8 inactivation, $K_i$ is the inhibitor concentration that
supports half the maximal rate of enzyme inactivation, $k_e$ is the first-order degradation rate constant of CYP2C8, $[I]_h$ is the unbound inhibitor concentration at the enzyme site in hepatocytes, and $f_{m,CYP2C8}$ is the fraction of repaglinide dose metabolised by CYP2C8. The $[I]_h$ was expressed on the basis of the observed total inhibitor plasma concentrations using the equation $[I]_h = C_{h,u}/C_{p,\text{tot}} \cdot C_{\text{avg,10 h}}$, where $C_{h,u}/C_{p,\text{tot}}$ is the hepatocyte (unbound) to plasma (total) concentration ratio and $C_{\text{avg,10 h}}$ is the average plasma concentration of gemfibrozil 1-O-β-glucuronide calculated from its AUC$_{0-10\ h}$. The $C_{h,u}/C_{p,\text{tot}}$ and $f_{m,CYP2C8}$ were left as the unknown parameters to be predicted with non-linear regression analysis. For this model, fixed values of the $k_{\text{inact}}$ (0.21 min$^{-1}$) and $K_i$ (20 µmol/l) were taken from a previous in vitro study (Ogilvie et al., 2006), and the $k_e$ (0.000558 min$^{-1}$) was obtained from a previous in vivo study (Backman et al., 2009). As this approach was found to best explain the interaction, the model was also applied to each subject individually.

To evaluate whether competitive inhibition of CYP2C8 or OATP1B1 by parent gemfibrozil or competitive inhibition of OATP1B1 by gemfibrozil 1-O-β-glucuronide could explain the observed drug interaction, the data were modelled using the competitive inhibition based equation $AUC_i/AUC_c = 1/[(f_{m,CYP2C8}/(1+[I]_h/K_i))+(1-f_{m,CYP2C8})]$ for inhibition of CYP2C8 and $AUC_i/AUC_c = 1/[(f_{\text{OATP1B1}}/(1+[I]_h/K_i))+(1-f_{\text{OATP1B1}})]$ for inhibition of OATP1B1, where $[I]_h$ is calculated as above for either gemfibrozil or gemfibrozil 1-O-β-glucuronide, $K_i$ is the in vitro competitive inhibition constant of the inhibitor, and $f_{\text{OATP1B1}}$ is the fraction transported by OATP1B1 (determines the maximal fold increase in repaglinide AUC, obtained with complete inhibition of OATP1B1). For this analysis, the $K_i$ of gemfibrozil for CYP2C8 (36.4 µmol/l) was taken from Wang et al. (Wang et al., 2002), using correction for microsomal binding of gemfibrozil, as described by Hinton et al. (Hinton et al., 2008),
and the $K_i$ values of gemfibrozil and gemfibrozil 1-O-$\beta$-glucuronide for inhibition of OATP1B1 were taken as IC50/2, based on the IC50 values of 7.4 $\mu$mol/l for gemfibrozil and 24.3 $\mu$mol/l for gemfibrozil 1-O-$\beta$-glucuronide (Shitara et al., 2004; Hinton et al., 2008). For all the above models, the $[I]_h$ was alternatively expressed as $[I]_h = C_{h,u}/C_{p,tot} \cdot C_{max}$. Because the direct CYP2C8-inhibitory effect of gemfibrozil 1-O-$\beta$-glucuronide seems to be very weak (Ogilvie et al., 2006), competitive inhibition of CYP2C8 by gemfibrozil 1-O-$\beta$-glucuronide was not considered as a relevant mechanism for the interaction.

As the in vitro inhibitory potencies and plasma unbound fractions of gemfibrozil and gemfibrozil 1-O-$\beta$-glucuronide suggested that inhibition of OATP1B1 by gemfibrozil 1-O-$\beta$-glucuronide is the second most important mechanism for the increase in repaglinide AUC, a combined reversible OATP1B1 inhibition and time-dependent CYP2C8 inhibition model was applied, using the following equation: $AUC_i/AUC_c = [1/(f_m,CYP2C8/(1+(k_{inact}/K_i) *[I]_h/k_e))+1-f_m,CYP2C8)]/[1/(f_{i,OATP1B1}/(1+[I]_p/K_i))+(1-f_{i,OATP1B1})]$. As OATP1B1 is localised on the sinusoidal membrane of hepatocytes, the unbound plasma $C_{max}$ or $C_{avg,10 \, h}$ of gemfibrozil 1-O-$\beta$-glucuronide was used as the inhibitor concentration at the transporter site $[I]_p$, instead of $[I]_h$, for inhibition of OATP1B1, assuming a plasma unbound fraction of 0.115 for gemfibrozil 1-O-$\beta$-glucuronide (Shitara et al., 2004). All the data were analyzed with PASW for Windows, version 17.0 (SPSS Inc, Chicago, Ill.).
Results

Pharmacokinetic variables of parent repaglinide. Escalating doses of gemfibrozil had a dose-dependent effect on the pharmacokinetics of repaglinide. The mean AUC$_{0\rightarrow\infty}$ of repaglinide was increased 1.8-, 4.5-, 6.7- or 8.3-fold by a single gemfibrozil dose of 30 mg, 100 mg, 300 mg or 900 mg, respectively ($P < 0.001$; Fig. 1, Table 2). In addition, the $C_{\text{max}}$ of repaglinide was increased after all gemfibrozil doses used, i.e., 1.4-, 1.7-, 2.1- and 2.4-fold, respectively ($P < 0.05$; Table 2). The increase in the $t_{1/2}$ of repaglinide reached a 2.0-fold prolongation ($P < 0.001$) with the 900 mg gemfibrozil dose, while shorter but statistically significant prolongations were observed with the smaller gemfibrozil doses ($P < 0.05$; Fig. 1, Table 2).

Pharmacokinetic variables of repaglinide metabolites. The 900 mg dose of gemfibrozil abolished the formation of the CYP2C8-dependent repaglinide metabolite M4 in almost all subjects, and the exact pharmacokinetic variables for M4 in this phase could not be calculated (Fig. 2, Table 2). With gemfibrozil doses 100 mg and 300 mg, dose-dependent decreases in the $C_{\text{max}}$, AUC$_{0-3\text{ h}}$ and AUC$_{0-9\text{ h}}$ of M4 were seen. The smallest gemfibrozil dose 30 mg decreased the M4/repaglinide AUC$_{0-3\text{ h}}$ ratio ($P < 0.005$; Table 2), but had no effect on the other pharmacokinetic variables of M4.

The M2/repaglinide AUC$_{0-9\text{ h}}$ ratio was decreased by 40-80% by the different gemfibrozil doses ($P < 0.005$). With gemfibrozil doses of 100 mg and higher, the $C_{\text{max}}$ of M2 was decreased and $t_{1/2}$ was prolonged (Table 2). In parallel with the prolonged $t_{1/2}$, small (<1.6-fold) increases in the AUC$_{0\rightarrow\infty}$ of M2 were observed at the highest gemfibrozil doses.
With the doses of 100 mg, 300 mg and 900 mg, gemfibrozil dose-dependently increased the AUC$_{0-\infty}$ of M1 and prolonged its $t_{1/2}$, while there were no changes in these variables with the 30 mg gemfibrozil dose (Table 2). The metabolite M1 to repaglinide AUC ratios were significantly decreased in all gemfibrozil phases compared to the control ($P < 0.005$).

**Pharmacodynamics.** The minimum blood glucose concentration was significantly smaller when repaglinide was given after a gemfibrozil dose of 100 mg, 300 mg or 900 mg ($P < 0.05$) than when it was given in the control phase (Fig. 1, Table 3). The mean blood glucose concentration 0-3 hours and 0-9 hours after repaglinide intake were significantly decreased by the 300 mg and 900 mg gemfibrozil doses only ($P < 0.005$ and $P < 0.05$, respectively).

**Gemfibrozil and gemfibrozil 1-O-β-glucuronide pharmacokinetics.** There was a more than 30-fold difference in the mean plasma concentrations of gemfibrozil and gemfibrozil 1-O-β-glucuronide between the 30 mg and 900 mg doses of gemfibrozil (Fig. 3). The AUC values of gemfibrozil and its glucuronide increased slightly more than dose-proportionally (Fig. 3, Table 4). The nonlinearity was slightly greater for the glucuronide than for parent gemfibrozil, resulting in a dose-dependent increase in the glucuronide/gemfibrozil AUC-ratio (Table 4).

**Relationship between the plasma concentrations of gemfibrozil or its 1-O-β-glucuronide and the fold increase in the AUC of repaglinide.** A static model assuming that mechanism-based inhibition of CYP2C8 by gemfibrozil 1-O-β-glucuronide is the sole explanation for the interaction described the relationship
between the extent of the interaction (repaglinide AUCi/AUCc) and the C_{avg,10h} of
gemfibrozil 1-O-β-glucuronide after the different gemfibrozil doses in the whole
population (r^2 = 0.79). With this non-linear regression model, the unknown
parameters, i.e., the fraction of repaglinide dose metabolised by CYP2C8 (f_{m,CYP2C8})
and ratio of unbound hepatocyte concentration to total plasma concentration
(C_{h,u}/C_{p, tot}) of gemfibrozil 1-O-β-glucuronide were estimated at 89% and 0.24,
respectively (data not shown). The individual model-based estimates for the f_{m,CYP2C8}
of repaglinide and the C_{h,u}/C_{p, tot} of gemfibrozil 1-O-β-glucuronide averaged 89% ±
2% and 0.28 ± 0.15, respectively (Fig. 4, Table 1).

Comparative static models assuming that either competitive inhibition of
CYP2C8 or OATP1B1 by gemfibrozil (r^2 = 0.76) or competitive inhibition of
OATP1B1 (r^2 = 0.79) by gemfibrozil 1-O-β-glucuronide is the sole explanation for
the interaction gave an identical 89% estimate for the f_{m,CYP2C8} or f_{t,OATP1B1} of
repaglinide (data not shown). However, the estimates for the C_{h,u}/C_{p, tot} -ratio of
gemfibrozil or its glucuronide were at least 1-2 orders of magnitude higher than that
obtained with the mechanism-based CYP2C8 inhibition model. The estimated
C_{h,u}/C_{p, tot} -ratios were 65 and 6.6 for competitive inhibition of CYP2C8 and
OATP1B1 by gemfibrozil, i.e., about 10,000 and 1000 times higher, respectively,
than the unbound fraction of gemfibrozil in plasma, 0.65% (Shitara et al., 2004). For
competitive inhibition of OATP1B1 by gemfibrozil 1-O-β-glucuronide, the C_{h,u}/C_{p, tot} -
ratio was 56, i.e., about 500 times higher than its unbound fraction in plasma, 11.5%
(Shitara et al., 2004). The use of inhibitor C_{max} instead of C_{avg,10h} did not significantly
improve the fit of any of the above models (data not shown).

The best fit was obtained by a combined reversible OATP1B1 inhibition
and mechanism-based CYP2C8 inhibition model, including the C_{avg,10h} of gemfibrozil
1-O-β-glucuronide (with the C_{hu}/C_{p,tot} -ratio) for inhibition of CYP2C8 and the unbound plasma C_{max} of gemfibrozil 1-O-β-glucuronide for inhibition of OATP1B1 (r^2 = 0.81). With this model, the estimated C_{hu}/C_{p,tot} -ratio, f_{m,CYP2C8} and f_{t,OATP1B1} were 0.37, 84% and 94%, respectively (Fig. 5).

**Genotypes.** One subject was homozygous and one was heterozygous for the CYP2C8*3 allele, associated with increased repaglinide metabolism (Niemi et al., 2003b), while the other subjects had the CYP2C8*1/*1 genotype. Four subjects were heterozygous for the SLCO1B1*1B allele, associated with increased OATP1B1 activity (Kalliokoski et al., 2008b), and four were heterozygous for the SLCO1B1*15 allele, associated with reduced OATP1B1 activity (Kalliokoski et al., 2008c), with one subject being compound heterozygous for both variants (SLCO1B1*1B/*15 genotype). No differences in the extent of the interaction between the SLCO1B1 genotypes could be seen, but the observed extent of the interaction and the mechanism-based CYP2C8 inhibition model-derived f_{m,CYP2C8} of repaglinide were greatest in carriers of the CYP2C8*3 allele (P = 0.0007 and P = 0.003, respectively; Fig. 4-5).
Discussion

In this study, gemfibrozil dose-dependently increased the AUC of repaglinide and inhibited its metabolism in humans. Already 30 mg of gemfibrozil increased repaglinide AUC nearly 2-fold. An average 4.5- and 6.7-fold AUC-increase was reached with the 100 mg and 300 mg doses, indicating that the dose-dependency was steep at the low dose range. With the highest 900 mg gemfibrozil dose, the M4 metabolite of repaglinide was completely abolished and the AUC of repaglinide was increased 8.3-fold, i.e., even more than previously with repeated doses of 600 mg gemfibrozil twice daily (Niemi et al., 2003a; Kalliokoski et al., 2008a; Tornio et al., 2008; Backman et al., 2009). Our regression models were consistent with over 90% inhibition of CYP2C8 by gemfibrozil at this dose level in all subjects and approximately 50% inhibition already with the 30 mg dose.

The metabolism of repaglinide occurs in the hepatocytes by CYP3A4 and CYP2C8 (Bidstrup et al., 2003; Kajosaari et al., 2005a; Kajosaari et al., 2005b). The uptake transporter OATP1B1 is involved in the transport of repaglinide from blood to hepatocytes (Niemi et al., 2005; Kalliokoski et al., 2008a; Kalliokoski et al., 2008b; Kalliokoski et al., 2008c). Although CYP2C8 seems to be more important than CYP3A4 in vivo (Niemi et al., 2003a), the exact contributions of CYP2C8 and CYP3A4 to repaglinide metabolism are not known. The main mechanism of the gemfibrozil-repaglinide interaction is thought to be mechanism-based inactivation of CYP2C8 by gemfibrozil 1-O-β-glucuronide (Ogilvie et al., 2006; Baer et al., 2009). In vitro, gemfibrozil 1-O-β-glucuronide has inhibited CYP2C8 with a k_{inact} of 0.21 min^{-1} and K_{I} of 20 or 52 μmol/l, depending on the microsomal protein concentration (0.1 or 1.0 mg/ml) used (Ogilvie et al., 2006). The inactivation of CYP2C8 occurs rapidly in vivo, reaching strong inhibition within 1 to 3 h after a single 600 mg gemfibrozil dose.
Gemfibrozil 1-\(\text{O-}\beta\)-glucuronide has also inhibited OATP1B1 activity in vitro, with an IC50 of 24.3 \(\mu\text{mol/l}\) (Shitara et al., 2004). Moreover, parent gemfibrozil has inhibited CYP2C8 competitively with a protein binding-corrected \(K_i\) of 36.4 \(\mu\text{mol/l}\) (Wang et al., 2002; Hinton et al., 2008) and OATP1B1 with an IC50 of 7.4-25 \(\mu\text{mol/l}\) (Ho et al., 2006; Hinton et al., 2008).

We applied enzyme/transporter inhibitory models to explain the relationship between the plasma concentrations of gemfibrozil or its 1-\(\text{O-}\beta\)-glucuronide and the increase in repaglinide AUC. With the models where mechanism-based inactivation of CYP2C8 by gemfibrozil 1-\(\text{O-}\beta\)-glucuronide was the main explanation for the interaction, we estimated that the unbound concentration of the glucuronide at the enzyme site in hepatocytes is lower than the total concentration of gemfibrozil 1-\(\text{O-}\beta\)-glucuronide in plasma (Shitara et al., 2004). Given that the glucuronide may accumulate in hepatocytes (Sallustio et al., 1996) and its peak concentrations are 2-5 times higher than its average plasma concentrations, our findings indicate that the mechanism-based CYP2C8 inhibitory effect is sufficiently strong to explain the majority of the interaction between gemfibrozil and repaglinide. With the competitive inhibition models, the estimated \(C_{\text{h,u}}/C_{\text{p,tot}}\) –ratios of gemfibrozil and its glucuronide were 500-10,000 times higher than the respective plasma unbound fractions, indicating that their competitive CYP2C8 and OATP1B1 inhibitory effects cannot alone explain the increases in the AUC of repaglinide. As a competitive OATP1B1 inhibition has previously been suggested to contribute to the interactions caused by gemfibrozil, it was incorporated into a model with mechanism-based inactivation. This model verified the crucial role of CYP2C8 inactivation by gemfibrozil 1-\(\text{O-}\beta\)-glucuronide in the
interaction, and suggested that inhibition of OATP1B1 is involved in the interaction mainly at gemfibrozil doses exceeding 300 mg and does not exceed 50% at clinically used gemfibrozil doses.

The combined CYP2C8 inactivation and OATP1B1 inhibition model resulted in an estimated “average” fraction of repaglinide dose metabolised by CYP2C8 of 84% and fraction transported by OATP1B1 of 94%. The estimated contribution of CYP2C8 is consistent with in vivo studies on interactions of repaglinide with CYP2C8 inhibitors (Niemi et al., 2003a; Backman et al., 2009), but higher than that suggested on the basis of in vitro studies (Bidstrup et al., 2003; Kajosaari et al., 2005a).

Unfortunately, individual estimates of the $f_{m,CYP2C8}$ of repaglinide could only be obtained with the mechanism-based CYP2C8 inhibition model (without OATP1B1).

Interestingly, the individual $f_{m,CYP2C8}$ values of repaglinide and the extent of the interaction were greatest in the two carriers of the $CYP2C8*3$ allele. Although the number of subjects in the present study was too small to draw any definitive conclusions, this finding is consistent with previous studies where $CYP2C8*3$ was associated with increased clearance of repaglinide (Niemi et al., 2003b; Niemi et al., 2005) and argues against the lack of association reported in other studies (Bidstrup et al., 2006; Tomalik-Scharte et al., 2011). The estimated $f_{t,OATP1B1}$ of repaglinide is also in line with previous pharmacogenetic studies. There are genotypes with increased or decreased OATP1B1 activity, and the differences in the AUC of repaglinide between the extreme genotypes are about 3-fold (Niemi et al., 2005; Kalliokoski et al., 2008a; Kalliokoski et al., 2008b; Kalliokoski et al., 2008c).

The AUC values of gemfibrozil and gemfibrozil 1-O-β-glucuronide increased slightly more than dose-proportionally (Fig. 3). The nonlinearity is apparently not clinically significant, as it required a 30-fold gemfibrozil dose range.
Yet, it may indicate some degree of saturation of transporter or enzyme mediated elimination of gemfibrozil or its glucuronide.

The fold-increase in repaglinide AUC approached a maximum with the 900 mg gemfibrozil dose (Fig. 4). This indicates that repaglinide metabolism was completely shifted to alternative routes, i.e., CYP2C8 was almost completely inactivated. Accordingly, the relative contribution of the CYP3A4-mediated metabolism of repaglinide, e.g., formation of M1 (Fig. 2), increased along with increasing doses of gemfibrozil and increasing concentrations of its 1-O-β-glucuronide. This explains why concomitant administration of gemfibrozil and the CYP3A4 inhibitor itraconazole has increased the AUC of repaglinide up to 19-fold, i.e., much more than did either of them alone (Niemi et al., 2003a).

The enzyme/transporter inhibition models including mechanism-based CYP2C8 inhibition were consistent with at least 50% inhibition of CYP2C8 with the 30 mg gemfibrozil dose, 75% inhibition at the 100 mg dose, 90% inhibition at the 300 mg dose and over 95% inhibition at the 900 mg dose. In individual subjects, the mechanism-based inhibition model suggested that >90% inhibition of CYP2C8 was achieved with the 300 mg dose in 9 of the 10 subjects.

In the present study, only a small 0.25 mg dose of repaglinide, used as the CYP2C8 model substrate, was given for safety reasons. The relative roles of CYP2C8 and CYP3A4 may be slightly different with higher 0.5-4 mg doses of repaglinide (Bidstrup et al., 2003; Kajosaari et al., 2005a; Bidstrup et al., 2006; Kalliokoski et al., 2008c). In any case, the consequences of CYP2C8 inhibition depend on the therapeutic index of the victim drug and the significance of CYP2C8 in its elimination. If other potential routes of elimination of CYP2C8 substrates are blocked by other drugs, or
other routes are not functional, e.g., due to genetic factors, the inhibition of CYP2C8 enzyme may cause an unusually strong interaction.

To conclude, the interaction between gemfibrozil and repaglinide is dose-dependent, with an incremental change in repaglinide plasma AUC and glucose lowering effect and reduction in repaglinide metabolite formation with incremental single doses of 30 mg, 100 mg, 300 mg and 900 mg gemfibrozil. The results are consistent with over 90% and 95% inhibition of hepatic CYP2C8 activity already with the 300 mg and 900 mg gemfibrozil doses. In clinical drug-interaction studies, a single 900 mg dose of gemfibrozil could be used instead of multiple gemfibrozil doses to achieve strong and rapid inhibition of CYP2C8 activity.
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Authorship contributions

*Participated in research design:* Honkalammi, Niemi, Neuvonen, and Backman.

*Conducted experiments:* Honkalammi, Niemi, Neuvonen, and Backman.

*Performed data analysis:* Honkalammi, Niemi, Neuvonen, and Backman.

*Wrote or contributed to the writing of the manuscript:* Honkalammi, Niemi, Neuvonen, and Backman.
References


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Requests for reprints:

Janne T. Backman, MD

PO Box 705, FI-00029 HUS, Finland

E-mail: janne.backman@helsinki.fi
LEGENDS FOR FIGURES

FIG. 1. Mean ± SD plasma concentrations of repaglinide and blood glucose concentrations in 10 healthy volunteers after a single oral dose of 0.25 mg repaglinide, which was administered 1 hour after placebo (control) or a single oral dose of 30, 100, 300 or 900 mg gemfibrozil. Open circles, control phase (no gemfibrozil); solid stars, gemfibrozil 30 mg; solid triangles, gemfibrozil 100 mg; solid squares, gemfibrozil 300 mg; solid circles, gemfibrozil 900 mg. Inset depicts the same data on a semi-logarithmic scale. For clarity, some error bars have been omitted.

FIG. 2. Mean plasma concentrations of repaglinide metabolites M1, M2 and M4 in 10 healthy volunteers after a single oral dose of 0.25 mg repaglinide, which was administered 1 hour after placebo (control) or a single oral dose of 30, 100, 300 or 900 mg gemfibrozil. Open circles, control phase (no gemfibrozil); solid stars, gemfibrozil 30 mg; solid triangles, gemfibrozil 100 mg; solid squares, gemfibrozil 300 mg; solid circles, gemfibrozil 900 mg.

FIG. 3. Mean ± SD plasma concentrations of gemfibrozil and gemfibrozil 1-O-β-glucuronide in 10 healthy volunteers after a single oral dose of 30, 100, 300 or 900 mg gemfibrozil (left panel). Solid stars, gemfibrozil 30 mg; solid triangles, gemfibrozil 100 mg; solid squares, gemfibrozil 300 mg; solid circles, gemfibrozil 900 mg. Relationship between the AUC$_{0-\infty}$ of gemfibrozil and gemfibrozil 1-O-β-glucuronide and gemfibrozil dose (right panel). The solid triangles depict individual data points and the line depicts the best fit equation derived by non-linear regression analysis, as described in Methods.
FIG. 4. Relationship between the fold increase in the $AUC_{0-\infty}$ of repaglinide and the average plasma concentration ($C_{avg,10h}$) of gemfibrozil 1-O-ß-glucuronide in 10 healthy subjects, when 0.25 mg repaglinide was given 1 hour after a single 30 mg, 100 mg, 300 mg or 900 mg dose of gemfibrozil. A mechanism-based CYP2C8 inhibition model was fitted to the individual data, as described in Methods.

Abbreviations: $AUC_{i}/AUC_c$, fold change in repaglinide area under the concentration-time curve; $C_{avg,10h}$, average plasma concentration calculated from $AUC_{0-10h}$; $f_{m,CYP2C8}$, fraction of repaglinide dose metabolised by CYP2C8, estimated by nonlinear regression analysis; $C_{h,u}/C_{p,tot}$, ratio of unbound hepatic to total plasma concentration of gemfibrozil 1-O-ß-glucuronide, estimated by nonlinear regression analysis. The curved lines represent the best-fit functions, the dotted lines represent the $AUC_{i}/AUC_c$-ratio when 50% of CYP2C8 is inactivated, and the dash-dot-dot lines represent the $AUC_{i}/AUC_c$-ratio when 90% of CYP2C8 is inactivated.

FIG. 5. Relationship of the increase in the $AUC_{0-\infty}$ of repaglinide ($AUC_{i}/AUC_c$) with the average plasma concentration ($C_{avg,10h}$) and peak plasma concentration ($C_{max}$) of gemfibrozil 1-O-ß-glucuronide, when 0.25 mg repaglinide was given 1 hour after a single 30 mg, 100 mg, 300 mg or 900 mg dose of gemfibrozil to 10 healthy subjects. Subjects carrying the $CYP2C8*3$ allele are indicated by red triangles (triangle pointing down indicates the homozygotic carrier). A combined competitive OATP1B1 inhibition (based on the unbound $C_{max}$ of gemfibrozil 1-O-ß-glucuronide) and mechanism-based CYP2C8 inhibition (based on the $C_{avg,10h}$ of gemfibrozil 1-O-ß-glucuronide) model was fitted to the data using published inhibitory constant values, as described in Methods. The curved contour depicts the best-fit model. Regression-
derived parameter values are shown with standard error. Abbreviations: AUC

/AUC_c, fold change in total area under the concentration-time curve of repaglinide; C_{avg,10h}, average plasma concentration calculated from AUC_{0-10h}; C_{h,u}/C_{p,tot}, ratio of unbound hepatic concentration to total plasma concentration; C_{max}, maximum plasma concentration; f_{m,CYP2C8}, fraction of dose metabolised by CYP2C8; k_{inact}, maximal rate of CYP2C8 inactivation; K_i, the inhibitor concentration that supports half the maximal rate of enzyme inactivation; k_e, the first-order degradation rate constant of CYP2C8; f_{i,OATP1B1}, fraction of dose transported by OATP1B1; f_{u,p}, unbound fraction in plasma; r^2, coefficient of determination from the nonlinear regression analysis.
## TABLE 1

Characteristics of the subjects, parameter values from regression analysis based on a mechanism-based CYP2C8 inhibition model, and the observed maximum fold increase in repaglinide AUC$_{0-\infty}$, when 0.25 mg repaglinide was given to each subjects 1 hour after a single 30 mg, 100 mg, 300 mg or 900 mg dose of gemfibrozil, compared to placebo.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>CYP2C8 genotype</th>
<th>SLC01B1 genotype</th>
<th>Gemfibrozil 1-O-β-glucuronide $C_{h,u}/C_{p,tot}$</th>
<th>Repaglinide $f_{m,CYP2C8}$</th>
<th>Repaglinide maximum AUC/AUC$_{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>Male</td>
<td>64</td>
<td>21</td>
<td>*1/*1</td>
<td>*1A/*1A</td>
<td>0.325</td>
<td>0.880</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>Male</td>
<td>84</td>
<td>26</td>
<td>*1/*1</td>
<td>*1A/*15</td>
<td>0.063</td>
<td>0.880</td>
<td>6.0</td>
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<td>3</td>
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<td>Male</td>
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<td>22</td>
<td>*1/*1</td>
<td>*1A/*1B</td>
<td>0.330</td>
<td>0.886</td>
<td>8.2</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
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<td>21</td>
<td>*1/*1</td>
<td>*1A/*1B</td>
<td>0.189</td>
<td>0.865</td>
<td>7.0</td>
</tr>
<tr>
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<td>24</td>
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<td>*1/*1</td>
<td>*1A/*1A</td>
<td>0.228</td>
<td>0.911</td>
<td>9.9</td>
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<tr>
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<td>20</td>
<td>Female</td>
<td>67</td>
<td>24</td>
<td>*1/*1</td>
<td>*1A/*1B</td>
<td>0.118</td>
<td>0.886</td>
<td>7.6</td>
</tr>
<tr>
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<td>27</td>
<td>*1/*1</td>
<td>*1A/*15</td>
<td>0.501</td>
<td>0.897</td>
<td>8.9</td>
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<tr>
<td>8</td>
<td>21</td>
<td>Male</td>
<td>82</td>
<td>23</td>
<td>*3/*3</td>
<td>*1A/*15</td>
<td>0.239</td>
<td>0.915</td>
<td>10.8</td>
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<tr>
<td>9</td>
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<td>Male</td>
<td>88</td>
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<td>*1/*1</td>
<td>*1A/*1A</td>
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<td>0.838</td>
<td>6.0</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>Male</td>
<td>72</td>
<td>24</td>
<td>*1/*3</td>
<td>*1B/*15</td>
<td>0.334</td>
<td>0.915</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Mean ± SD 23 ± 2 73 ± 10 23 ± 2 0.285 ± 0.149 0.887 ± 0.024 8.4 ± 1.9
BMI, body mass index; $C_{u/Cp,tot}$, ratio of the unbound concentration in hepatocytes to the total concentration in plasma, according to nonlinear regression analysis as described in Methods; $f_{m,CYP2C8}$, the fraction of the dose metabolized by CYP2C8 in the liver, as estimated using nonlinear regression analysis; repaglinide maximum AUC/AUCc, individual observed maximal fold increase in repaglinide AUC in any gemfibrozil phase compared to control phase.
Table 2

Pharmacokinetic variables of repaglinide and its metabolites M1, M2 and M4 after a single oral dose of 0.25 mg repaglinide in 10 healthy volunteers, when repaglinide was administered 1 hour after placebo (control) or a single dose of 30, 100, 300 or 900 mg gemfibrozil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>30 mg dose</th>
<th>100 mg dose</th>
<th>300 mg dose</th>
<th>900 mg dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Repaglinide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>4.3 ± 1.2</td>
<td>5.6 ± 1.1^*</td>
<td>6.9 ± 1.9^**</td>
<td>8.8 ± 2.5^***^††</td>
<td>9.6 ± 2.2^***^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.4 (0.8-2.2)</td>
<td>1.7 (1.0-2.4)</td>
<td>2.1 (1.3-3.0)</td>
<td>2.4 (1.4-3.2)</td>
<td></td>
</tr>
<tr>
<td>tmax (min)</td>
<td>30 (30-45)</td>
<td>37.5 (30-45)</td>
<td>45 (30-100)^†</td>
<td>37.5 (30-80)</td>
<td>37.5 (30-60)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.3^*</td>
<td>1.9 ± 0.3^**</td>
<td>2.4 ± 0.4^***^†††</td>
<td>3.1 ± 0.6^***^†††^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.2 (0.9-1.7)</td>
<td>1.2 (0.9-1.4)</td>
<td>1.6 (1.1-2.0)</td>
<td>2.0 (1.4-2.9)</td>
<td></td>
</tr>
<tr>
<td>AUC0-9h (ng×h/ml)</td>
<td>4.7 ± 1.0</td>
<td>8.1 ± 2.1^***</td>
<td>20.2 ± 7.4^***^†††</td>
<td>28.7 ± 8.9^***^†††</td>
<td>33.1 ± 8.4^***^†††^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.8 (1.1-2.3)</td>
<td>4.3 (2.2-6.5)</td>
<td>6.2 (3.1-8.0)</td>
<td>7.2 (4.9-9.8)</td>
<td></td>
</tr>
<tr>
<td>AUC0-∞ (ng×h/ml)</td>
<td>4.7 ± 1.0</td>
<td>8.3 ± 2.3^***</td>
<td>21.2 ± 8.0^***^†††</td>
<td>31.5 ± 10.4^***^†††</td>
<td>38.8 ± 11.4^***^†††^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.8 (1.1-2.4)</td>
<td>4.5 (2.2-6.9)</td>
<td>6.7 (3.3-8.8)</td>
<td>8.3 (5.6-11.4)</td>
<td></td>
</tr>
<tr>
<td><strong>M1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>0.16 ± 0.06</td>
<td>0.17 ± 0.05</td>
<td>0.18 ± 0.07</td>
<td>0.24 ± 0.07^***^†††</td>
<td>0.27 ± 0.07^***^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.2 (0.6-2.1)</td>
<td>1.2 (0.5-2.3)</td>
<td>1.6 (1.0-2.6)</td>
<td>1.8 (1.1-2.7)</td>
<td></td>
</tr>
<tr>
<td>tmax (min)</td>
<td>52.5 (30-120)</td>
<td>45 (30-60)</td>
<td>45 (30-100)^†</td>
<td>45 (30-100)</td>
<td>60 (30-120)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>0.80 ± 0.16</td>
<td>0.9 ± 0.4</td>
<td>1.8 ± 0.5^***^†††</td>
<td>1.9 ± 0.4^***^†††</td>
<td>2.5 ± 0.4^***^†††^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.2 (0.7-2.2)</td>
<td>2.3 (1.2-3.8)</td>
<td>2.5 (1.3-3.9)</td>
<td>3.2 (2.1-4.8)</td>
<td></td>
</tr>
<tr>
<td>AUC0-9h (ng×h/ml)</td>
<td>0.26 ± 0.08</td>
<td>0.29 ± 0.08</td>
<td>0.49 ± 0.17^***^†††</td>
<td>0.75 ± 0.23^***^†††</td>
<td>1.04 ± 0.25^***^†††^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.2 (0.6-1.7)</td>
<td>2.0 (0.9-3.2)</td>
<td>3.1 (2.0-7.2)</td>
<td>4.2 (2.6-5.7)</td>
<td></td>
</tr>
<tr>
<td>AUC0-∞ (ng×h/ml)</td>
<td>0.27 ± 0.07</td>
<td>0.31 ± 0.08</td>
<td>0.55 ± 0.19^***^†††</td>
<td>0.80 ± 0.24^***^†††</td>
<td>1.16 ± 0.28^***^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.2 (0.6-1.6)</td>
<td>2.0 (1.0-3.0)</td>
<td>3.1 (2.1-6.8)</td>
<td>4.4 (2.8-5.6)</td>
<td></td>
</tr>
<tr>
<td>M1/repaglinide AUC0-9h ratio</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.01^***</td>
<td>0.03 ± 0.02^**</td>
<td>0.03 ± 0.01^†</td>
<td>0.03 ± 0.01^***</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>0.7 (0.3-1.0)</td>
<td>0.5 (0.2-0.8)</td>
<td>0.5 (0.3-0.9)</td>
<td>0.6 (0.5-0.8)</td>
<td></td>
</tr>
<tr>
<td><strong>M2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>0.15 ± 0.06</td>
<td>0.12 ± 0.04</td>
<td>0.09 ± 0.05^***</td>
<td>0.09 ± 0.03^**</td>
<td>0.11 ± 0.06^‡</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>0.9 (0.5-1.5)</td>
<td>0.6 (0.3-0.8)</td>
<td>0.6 (0.4-1.2)</td>
<td>0.8 (0.5-1.2)</td>
<td></td>
</tr>
<tr>
<td>tmax (min)</td>
<td>45 (30-80)</td>
<td>52.5 (30-100)</td>
<td>60 (30-100)</td>
<td>45 (30-100)</td>
<td>45 (30-100)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>0.9 ± 0.5</td>
<td>0.8 ± 0.2</td>
<td>2.0 ± 1.1^†††</td>
<td>2.4 ± 1.1^†††</td>
<td>2.1 ± 0.8^***</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.1 (0.3-1.8)</td>
<td>2.5 (1.1-5.2)</td>
<td>3.4 (5.5-5.9)</td>
<td>2.9 (5.5-5.0)</td>
<td></td>
</tr>
<tr>
<td>AUC0-9h (ng×h/ml)</td>
<td>0.24 ± 0.08</td>
<td>0.24 ± 0.09</td>
<td>0.23 ± 0.09</td>
<td>0.25 ± 0.06</td>
<td>0.35 ± 0.20^‡</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.1 (0.6-1.9)</td>
<td>1.1 (0.5-1.6)</td>
<td>1.1 (0.8-1.5)</td>
<td>1.5 (0.8-2.8)</td>
<td></td>
</tr>
<tr>
<td>AUC0-∞ (ng×h/ml)</td>
<td>0.25 ± 0.07</td>
<td>0.25 ± 0.09</td>
<td>0.28 ± 0.08</td>
<td>0.30 ± 0.07^†</td>
<td>0.41 ± 0.21^‡</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.0 (0.6-1.7)</td>
<td>1.2 (0.5-1.7)</td>
<td>1.2 (0.9-1.7)</td>
<td>1.6 (1.0-3.0)</td>
<td></td>
</tr>
<tr>
<td>M2/repaglinide AUC0-9h ratio</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.01^***</td>
<td>0.01 ± 0.01^***</td>
<td>0.01 ± 0.01^***</td>
<td>0.404^***^†††</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>0.6 (0.3-1.2)</td>
<td>0.3 (0.1-0.5)</td>
<td>0.2 (0.1-0.3)</td>
<td>0.2 (0.1-0.3)</td>
<td></td>
</tr>
<tr>
<td><strong>M4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (U/ml)</td>
<td>3.5 ± 1.4</td>
<td>3.3 ± 1.5</td>
<td>1.9 ± 1.6^***</td>
<td>1.1 ± 1.3^***</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.0 (0.5-1.4)</td>
<td>0.5 (0.1-1.0)</td>
<td>0.3 (0.1-0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC0-9h (U×h/ml)</td>
<td>AUC0-3h (U×h/ml)</td>
<td>M4/ repaglinide AUC0-9h ratio</td>
<td>M4/ repaglinide AUC0-3h ratio</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5 ± 1.2</td>
<td>4.0 ± 1.2</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Fold (range)</td>
<td>1.0 (0.7-1.3)</td>
<td>0.6 (0.6-1.4)</td>
<td>0.62 (0.41-1.03)</td>
<td>0.66 (0.35-1.14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.8 ± 1.6</td>
<td>4.2 ± 1.5</td>
<td>0.76 ± 0.29**</td>
<td>0.66 ± 0.27**</td>
<td></td>
</tr>
<tr>
<td>Fold (range)</td>
<td>0.8 (0.4-1.3)</td>
<td>0.6 (0.02-1.3)</td>
<td>0.26 ± 0.18**</td>
<td>0.23 ± 0.21***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4 ± 2.3†</td>
<td>2.7 ± 2.2†</td>
<td>0.23 ± 0.18**</td>
<td>0.11 ± 0.12***</td>
<td></td>
</tr>
<tr>
<td>Fold (range)</td>
<td>0.5 (0.3-0.9)</td>
<td>0.4 (0.1-0.8)</td>
<td>0.02 (0.09-0.49)</td>
<td>0.06 (0.06-0.60)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0 ± 1.2***</td>
<td>1.5 ± 1.2***</td>
<td>0.12 ± 0.10**</td>
<td>0.11 ± 0.12***</td>
<td></td>
</tr>
<tr>
<td>Fold (range)</td>
<td>0.5 (0.3-0.9)</td>
<td>0.4 (0.1-0.8)</td>
<td>0.02 (0.09-0.49)</td>
<td>0.06 (0.06-0.60)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>0.12 ± 0.10**</td>
<td>0.11 ± 0.12***</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05 (0.04-0.29)</td>
<td>0.06 (0.06-0.60)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D.; AUC0-3h, area under the plasma concentration-time curve from time 0 to 3 hours; AUC0-9h, area under the plasma concentration-time curve from time 0 to 9 hours; AUC0-∞, area under the plasma concentration-time curve from time 0 to infinity; C_{max}, peak plasma concentration; t_{max}, time to peak concentration; t_{1/2}, elimination half-life. * P<0.05 vs. control, ** P<0.005 vs. control, *** P<0.001 vs. control, † P<0.05 vs. 30 mg, †† P<0.005 vs. 30 mg, ††† P<0.001 vs. 30 mg, ‡ P<0.005 vs. 30 mg, ‡‡ P<0.005 vs. 100 mg, ‡‡‡ P<0.001 vs. 100 mg, ¶ P<0.05 vs. 300 mg, ¶¶ P<0.005 vs. 300 mg, ¶¶¶ P<0.005 vs. 300 mg.
### TABLE 3

**Blood glucose levels in 10 healthy volunteers after a single oral dose of 0.25 mg repaglinide,**

*which was administered 1 hour after placebo or a single oral dose of 30, 100, 300 or 900 mg gemfibrozil.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>30 mg</th>
<th>100 mg</th>
<th>300 mg</th>
<th>900 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline concentration (mmol/l)</td>
<td>5.1 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>5.2 ± 0.8</td>
<td>5.0 ± 0.6</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Minimum concentration (mmol/l)</td>
<td>3.6 ± 0.4</td>
<td>3.5 ± 0.6</td>
<td>3.1 ± 0.5*</td>
<td>3.3 ± 0.5*</td>
<td>2.7 ± 0.6*** ††, ¶</td>
</tr>
<tr>
<td>Mean concentration from 0 to 3 h (mmol/l)</td>
<td>4.9 ± 0.5</td>
<td>4.7 ± 0.5</td>
<td>4.5 ± 0.3†</td>
<td>4.4 ± 0.4**</td>
<td>4.3 ± 0.5*** ††, ¶</td>
</tr>
<tr>
<td>Mean concentration from 0 to 9 h (mmol/l)</td>
<td>4.8 ± 0.4</td>
<td>4.8 ± 0.4</td>
<td>4.6 ± 0.3†</td>
<td>4.4 ± 0.4**</td>
<td>4.2 ± 0.5*** ††</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * P < 0.05 vs. control, ** P < 0.005 vs. control, *** P < 0.001 vs. control, † P < 0.05 vs. 30 mg, †† P < 0.005 vs. 30 mg, ¶ P < 0.05 vs. 300 mg.
TABLE 4

Pharmacokinetic variables of gemfibrozil and gemfibrozil 1-O-β-glucuronide in 10 healthy volunteers after a single dose of 30, 100, 300 or 900 mg gemfibrozil, which was taken 1 hour before administration of repaglinide.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gemfibrozil dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 mg</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td></td>
</tr>
<tr>
<td>$C_{1h}$ (μg/ml)</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>$t_{max}$ (min)</td>
<td>60 (30-105)</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>$AUC_{0-10h}$ (μg×h/ml)</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg×h/ml)</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Gemfibrozil-1-O-β-glucuronide</td>
<td></td>
</tr>
<tr>
<td>$C_{1h}$ (μg/ml)</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>$t_{max}$ (min)</td>
<td>67.5 (30-120)</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>$AUC_{0-10h}$ (μg×h/ml)</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg×h/ml)</td>
<td>0.95 ± 0.17</td>
</tr>
<tr>
<td>Glucuronide/gemfibrozil $AUC_{0-\infty}$ ratio</td>
<td>0.47 ± 0.08</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.; except for $t_{max}$ data, which are given as median and range. $C_{1h}$, plasma concentration 1 hour after gemfibrozil intake (i.e., at the time of repaglinide administration); $C_{max}$, peak plasma concentration; $t_{max}$, time-to-peak plasma concentration; $t_{1/2}$, elimination half-life; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; $AUC_{0-10h}$, area under the plasma concentration-time curve from time 0 to 10 hours. Statistical comparisons were performed only for $t_{max}$, $t_{1/2}$ and glucuronide/gemfibrozil $AUC_{0-\infty}$ ratio data. * $P<0.05$ vs. 30 mg, *** $P<0.001$ vs. 30 mg, † $P<0.05$ vs. 100 mg.
Figure 3

AUC = e^\alpha \cdot \text{dose}^\beta

\alpha = -3.41 (95% CI: -3.89, -3.12)
\beta = 1.21 (95% CI: 1.15, 1.26)

AUC = e^\alpha \cdot \text{dose}^\beta

\alpha = -4.51 (95% CI: -4.80, -4.22)
\beta = 1.33 (95% CI: 1.28, 1.39)
Figure 4

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Gemfibrozil 1-O-β-glucuronide
\[ \frac{AUC_i}{AUC_c} = \left( \frac{1}{f_{m,\text{CYP2C8}} \left( 1 + \frac{K_{\text{inact}}}{K_i} \cdot \left( \frac{L_i}{L_e} \right) \right) + 1 - f_{m,\text{CYP2C8}}} \right) \cdot \left( \frac{1}{f_{t,\text{OATP1B1}} \left( 1 + \frac{L_i}{L_e} / K_i \right) + 1 - f_{t,\text{OATP1B1}}} \right) \]

\[ \frac{L_i}{L_e} = \left( \frac{C_{h,u}}{C_{p,tot}} \right) \cdot C_{\text{avg,10h}} \quad \frac{L_i}{L_e} = f_{u,p} \cdot C_{\text{max}} \]

- \( C_{h,u} / C_{p,tot} = 0.369 \pm 0.137 \)
- \( f_{m,\text{CYP2C8}} = 84.1\% \pm 2.4\% \)
- \( f_{t,\text{OATP1B1}} = 93.7\% \pm 31.0\% \)
- \( r^2 = 0.811 \)