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Impact of the *CYP2C8 *3* polymorphism on the drug–drug interaction between gemfibrozil and pioglitazone

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- A drug–drug interaction exists between gemfibrozil (CYP2C8 inhibitor) and pioglitazone (CYP2C8 substrate), whereby gemfibrozil increases pioglitazone plasma exposure. Substantial interindividual variability exists in the pharmacokinetic magnitude of this drug–drug interaction.
- *CYP2C8 *3* is associated with increased metabolism and decreased plasma exposure of pioglitazone.
- Polymorphisms in CYP metabolizing enzyme genes, namely *CYP2C19* and *CYP2D6*, have been shown to influence the magnitude of inhibitory drug–drug interactions. However, the extent to which *CYP2C8* polymorphisms (e.g. *CYP2C8 *3*) affect the interaction between gemfibrozil and pioglitazone is not known.

WHAT THIS STUDY ADDS

- The *CYP2C8 *3* allele influences pharmacokinetic variability in the drug–drug interaction between gemfibrozil and pioglitazone. *CYP2C8 *3* carriers experienced a larger relative increase in pioglitazone plasma exposure following gemfibrozil administration than wild-type homozygotes.
- Consideration should be given to the contribution of polymorphic *CYP2C8* alleles to interindividual variability in the pharmacokinetic magnitude of CYP2C8-mediated drug–drug interactions.

AIM

The objective of this study was to determine the extent to which the *CYP2C8 *3* allele influences pharmacokinetic variability in the drug–drug interaction between gemfibrozil (CYP2C8 inhibitor) and pioglitazone (CYP2C8 substrate).

METHODS

In this randomized, two phase crossover study, 30 healthy Caucasian subjects were enrolled based on *CYP2C8 *3* genotype ($n = 15$, *CYP2C8 *1/*1*; $n = 15$, *CYP2C8 *3* carriers). Subjects received a single 15 mg dose of pioglitazone or gemfibrozil 600 mg every 12 h for 4 days with a single 15 mg dose of pioglitazone administered on the morning of day 3. A 48 h pharmacokinetic study followed each pioglitazone dose and the study phases were separated by a 14 day washout period.

RESULTS

Gemfibrozil significantly increased mean pioglitazone AUC(0,∞) by 4.3-fold ($P < 0.001$) and there was interindividual variability in the magnitude of this interaction (range, 1.8- to 12.1-fold). When pioglitazone was administered alone, the mean AUC(0,∞) was 29.7% lower ($P = 0.01$) in *CYP2C8 *3* carriers compared with *CYP2C8 *1* homozygotes. The relative change in pioglitazone plasma exposure following gemfibrozil administration was significantly influenced by *CYP2C8* genotype. Specifically, *CYP2C8 *3* carriers had a 5.2-fold mean increase in pioglitazone AUC(0,∞) compared with a 3.3-fold mean increase in *CYP2C8 *1* homozygotes ($P = 0.02$).

CONCLUSION

*CYP2C8 *3* is associated with decreased pioglitazone plasma exposure *in vivo* and significantly influences the pharmacokinetic magnitude of the gemfibrozil–pioglitazone drug-drug interaction. Additional studies are needed to evaluate the impact of *CYP2C8* genetics on the pharmacokinetics of other CYP2C8-mediated drug–drug interactions.

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Introduction

Drug–drug interactions involving inhibition or induction complicate the management of cardiometabolic diseases and interindividual variability exists in the pharmacokinetic magnitude of these interactions. There is increasing evidence that genetic variation influences the extent of drug–drug interactions, particularly those involving cytochrome P450 (CYP) metabolizing enzymes [1]. An example of an inhibitory drug–drug interaction that is germane to cardiometabolic pharmacotherapy is the gemfibrozil-mediated CYP2C8 inhibition of pioglitazone metabolism.

CYP2C8 plays an important role in the hepatic metabolism of numerous pharmacologic agents including pioglitazone (thiazolidinedione), repaglinide (meglitinide), cerivastatin (HMG-CoA reductase inhibitor) and paclitaxel (chemotherapeutic agent) [2, 3]. Pioglitazone, a peroxisome proliferator-activated receptor-γ agonist, is indicated for the treatment of type 2 diabetes. It is hepatically metabolized by CYP2C8, and to a lesser extent by CYP3A4, CYP1A2, CYP2C9 and CYP2D6 [4–7]. Gemfibrozil, a fibric acid derivative used in the treatment of hypertriglyceridaemia, potently inhibits CYP2C8 *in vitro* and *in vivo* [8–17]. Two clinical studies have shown that gemfibrozil increases pioglitazone plasma exposure approximately 3-fold due to CYP2C8 inhibition [5, 18]. Notably, substantial interindividual variability exists in the magnitude of this interaction, with increases in pioglitazone plasma exposure ranging from 2.3-fold to 6.5-fold [5, 18]. Previous studies have shown that polymorphisms in *CYP* genes influence the magnitude of CYP-mediated inhibitory drug–drug interactions [1]. For example, the extent of CYP2C19- and CYP2D6-mediated inhibition tends to be greater in extensive *vs.* poor metabolizers [19–23]. To our knowledge, the impact of *CYP2C8* polymorphisms on the drug–drug interaction between gemfibrozil and pioglitazone has not been prospectively evaluated in clinical studies.

*CYP2C8 *3* is the most commonly studied functional polymorphism in *CYP2C8*. The *CYP2C8 *3* allele is comprised of two highly linked nonsynonymous polymorphisms, Arg139Lys and Lys399Arg, in exons 3 and 8, respectively. *CYP2C8 *3* is common in Caucasians (10% to 23%) but is rare in African and Asian populations [2, 3, 24]. There are conflicting *in vitro* data regarding the effect of *CYP2C8 *3* on metabolic activity, with reports of increased, decreased or no change in metabolism [7, 24–30]. *In vivo*, the consequences of *CYP2C8 *3* also appear to be substrate-dependent, with increased metabolism of agents such as pioglitazone, rosiglitazone and repaglinide, but decreased metabolism of R-ibuprofen [31–36]. In terms of the clinical pharmacokinetics of pioglitazone, a healthy volunteer study showed that carriers of the *CYP2C8 *3* allele had lower pioglitazone plasma exposure and a higher rate of metabolite formation than subjects with the *CYP2C8 *1/*1* genotype [31].

Given the known influence of *CYP2C8 *3* on pioglitazone pharmacokinetics, the objective of this study was to determine the extent to which *CYP2C8 *3* influences interindividual pharmacokinetic variability in the drug–drug interaction between gemfibrozil and pioglitazone in healthy volunteers.

Methods

Participants

The study was approved by the Colorado Multiple Institutional Review Board and all subjects provided written informed consent. The study consisted of healthy Caucasian men and women between 21 to 60 years of age. Participants were prospectively screened and stratified according to *CYP2C8* genotype as follows: Group 1 = *CYP2C8 *1/*1* genotype (reference); Group 2 = carriers of at least one copy of the *CYP2C8 *3* allele (i.e. **1/*3* or **3/*3*). Participants were excluded from the study for any of the following: body mass index <18 kg m^{-2} or ≥35 kg m^{-2}, current or past history of cardiovascular, hepatic, renal, endocrine, gastrointestinal, haematologic, immunologic, or neurologic diseases, history of rhabdomyolysis, active malignancy, self-reported HIV positivity, active drug or alcohol abuse or pregnancy or lactation. Laboratory exclusion criteria included fasting plasma glucose ≥126 mg dl^{-1}, serum potassium >5 mEq l^{-1} or <3.3 mEq l^{-1}, serum creatinine >1.2 mg dl^{-1}, liver function tests ≥ two times the upper limit of normal, haematocrit <36% in men or <34% in women, platelets <150 × 10^9 l^{-1}, white blood cell count <4.0 × 10^9 l^{-1} or >11.1 × 10^9 l^{-1}, or any other laboratory abnormalities classified as grade 2 or higher per published grading criteria [37]. Subjects were also excluded for concomitant use of any of the following: antidiabetic medications, statins, fibrates, systemic glucocorticoids and/or any other agent known to inhibit or induce the CYP2C8 and/or CYP3A4 metabolizing enzymes (e.g. trimethoprim, fluvoxamine, rifampicin, grapefruit juice).

Study design

The study was conducted in an open-label, randomized, two phase crossover design. In one phase, subjects received a single 15 mg dose of pioglitazone by mouth at 09.00 h. In the other phase, subjects received 600 mg of gemfibrozil by mouth at 08.00 h and 20.00 h for 4 days, with a single 15 mg dose of pioglitazone administered by mouth on day 3 at 09.00 h. The two phases were separated by a 14 day washout period. An intensive 48 h pharmacokinetic study was conducted after each pioglitazone dose. For the pharmacokinetic studies, subjects were admitted to the University of Colorado Denver Clinical and Translational Research Center (CTRC) Inpatient Unit after an overnight fast. In both phases, subjects received a standardized breakfast (600 calories; 55% carbohydrates, 15% protein and 30% fat) 2 h after pioglitazone ingestion. Subjects also

received meals 6, 10 and 24 h after pioglitazone dosing. All meals were caffeine-free and subjects were asked to abstain from caffeine and smoking during the 48 h period. Blood samples (5 ml in ethylene-diaminetetraacetic acid [EDTA]) were collected pre-dose and 1, 2, 3, 4, 5, 7, 9, 12, 18, 24 and 48 h post pioglitazone dosing in both phases. Plasma was harvested within 30 min of each blood draw and stored at –80°C until analytical processing.

Genetic analyses

For DNA collection during the screening process, subjects were asked to swish vigorously 15 ml of Scope® mouthwash (Procter and Gamble, Cincinnati, OH, USA) for 1 min and then expectorate into a sterile collection tube [38]. Genomic DNA was isolated from buccal cells using a commercially available kit (QIAmp DNA Mini Kit, Qiagen, Valencia, CA, USA). The nonsynonymous *CYP2C8* polymorphisms, Arg139Lys (rs11572080) and Lys399Arg (rs10509681), were genotyped using PCR-Pyrosequencing analysis (PSQ 96MA, Qiagen, Valencia, CA, USA) according to our previously published method [33]. Automated PSQ 96MA SNP software version 2.0 (Qiagen, Valencia, CA, USA) was used to make genotype determinations. *CYP2C8 *3* was denoted as the presence of the Lys and Arg alleles at codons 139 and 399, respectively.

Drug concentration analyses

Plasma concentrations of pioglitazone were measured with a validated LC/MS assay. The internal standard was deuterated pioglitazone (pioglitazone-d4; Toronto Research Chemicals, North York, Ontario, Canada). Pioglitazone was extracted from plasma using a liquid–liquid extraction procedure with t-butylmethylether at an acidic pH. Chromatographic separation was conducted on a 2.1 × 50 mm, 5 µm Sunfire C18 column (Waters Corporation, Milford, MA, USA) maintained at 40°C. The mobile phase consisted of 60% ammonium acetate (pH 4.5) : 40% acetonitrile (v/v) and was delivered at a flow rate of 300 µl min^{-1}. The retention time for pioglitazone was 3.5 min. A single quadrupole mass spectrometer (Thermo Fisher MSQ, Thermo Fisher, San Jose, CA, USA) was used in ESI, positive polarity mode. Analytes were detected using single ion monitoring mode, with pioglitazone and pioglitazone-d4 detected at *m/z* of 357.07 and 361.21, respectively. The needle voltage was set at 2.5 kV and cone voltage at 125 V. Nitrogen was used as the source gas and was maintained at 75 psi. Data acquisition and processing were performed using Xcalibur software, version 1.3 (Thermo Fisher, San Jose, CA, USA). Calculations were based on peak area ratios of analyte to internal standard. Concentrations were interpolated from a linear least squares regression calibration curve, based on 1/concentration weighting. The lower limit of quantification (LLOQ) of pioglitazone was 5 ng ml^{-1}, and the assay was linear over the range of 5 ng ml^{-1}–2000 ng ml^{-1}. Validation data for pioglitazone non-LLOQ samples were within ± 15% for inter- and intra-day accuracy and precision. The LLOQ data were within ± 20% for both accuracy and precision. Possible interference between pioglitazone, gemfibrozil and gemfibrozil 1-*O*-β-glucuronide was evaluated, but not observed, in this method. Plasma concentrations of gemfibrozil were measured with a validated LC/MS assay as previously described [39]. Deuterated gemfibrozil (gemfibrozil-d6) was used as the internal standard. The LLOQ of gemfibrozil was 0.5 µg ml^{-1} and the assay was linear over the range of 0.5 to 50 µg ml^{-1}. Inter- and intraday accuracy and precision were within ± 15% [39]. Gemfibrozil 1-*O*-β-glucuronide metabolite area ratios were determined from LC/MS chromatograms initially analyzed for gemfibrozil concentrations, which contained a glucuronide peak as a result of in-source dissociation, and were normalized against the internal standard, gemfibrozil-d6. Gemfibrozil 1-*O*-β-glucuronide concentrations were then determined from the gemfibrozil parent calibration curve, using 1/concentration weighting [39].

Pharmacokinetic analysis

Plasma concentration–time curves of pioglitazone, gemfibrozil and gemfibrozil 1-*O*-β-glucuronide were generated, and the maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were observed directly from these curves. Pharmacokinetic parameters were estimated by noncompartmental analysis using WinNonlin version 5.2.1 software (Pharsight Corporation, Mountain View, CA, USA). The terminal elimination rate constant (λ_z) was obtained by regression analysis of the log-linear portion of the concentration–time curves. Pioglitazone area under the plasma concentration–time curve from 0 to infinity (AUC(0,∞)), and gemfibrozil and gemfibrozil 1-*O*-β-glucuronide AUC from 0 to 10 h (AUC(0,10 h)) were calculated using the log linear trapezoidal rule. Pioglitazone and gemfibrozil half-life ($t_{1/2}$) were calculated from the following equation, $t_{1/2} = \ln(2)/\lambda_z$. Apparent oral clearance (CL/*F*) was calculated as dose (in mg)/AUC(0,∞). Weight-adjusted oral clearance (CL/*F* kg^{-1}) was calculated as [dose (mg)/AUC(0,∞)]/subject weight (kg).

Statistical analyses

The primary endpoint was the relative change in pioglitazone AUC(0,∞) between *CYP2C8* genotype groups (i.e., **1/*1 vs.*3* carriers). Secondary pharmacokinetic endpoints that were compared between *CYP2C8 *1* homozygotes and *CYP2C8 *3* carriers included: (i) other pioglitazone pharmacokinetic parameters (e.g., AUC(0,48 h), CL/*F* kg^{-1}, $t_{1/2}$) and (ii) gemfibrozil and gemfibrozil 1-*O*-β-glucuronide pharmacokinetic parameters.

Baseline demographics were compared between *CYP2C8 *1* homozygotes and *CYP2C8 *3* carriers by independent *t*-tests for continuous data, and Chi-square or Fisher's exact tests for categorical data. Non-normally distributed pharmacokinetic parameters (e.g. AUC and C_{max}) were log-transformed prior to analysis. In the entire study cohort, the changes in pioglitazone pharmacokinetic

Table 1

Baseline demographics ($n = 30$) by *CYP2C8* genotype group

Demographic variable	*CYP2C8 *1/*1* $n = 15$	*CYP2C8 *3* carriers $n = 15$	*P* value
Male, *n* (%)	4 (27%)	5 (33%)	0.99
Hispanic ethnicity, *n* (%)	0	2 (13%)	0.48
Current smoker, *n* (%)	3 (20%)	1 (7%)	0.60
Hormonal contraceptive, *n* (%)	4 (27%)	4 (27%)	–
Age (years)	35 ± 9	37 ± 12	0.45
Weight (kg)	74.5 ± 15.7	71.0 ± 16.4	0.56
Body mass index (kg m^{-2})	24.9 ± 3.0	25.3 ± 4.3	0.77

Data are presented as number (%) or mean ± SD. *P* values are for the comparison of baseline demographics between *CYP2C8* genotype groups. All subjects in the study classified their race as Caucasian.

Table 2

Single dose pharmacokinetics of pioglitazone 15 mg in all study participants ($n = 30$) when pioglitazone was administered alone and in combination with gemfibrozil

Pioglitazone pharmacokinetic parameter	Pioglitazone alone	Pioglitazone + gemfibrozil	Relative change [(Pioglitazone + gemfibrozil)/pioglitazone alone]	*P* value
C_{max} (ng ml^{-1})	608 ± 215	678 ± 187	1.3 (1.0, 1.6)	0.10
AUC(0,∞) (ng ml^{-1} h)	5 770 ± 2 840	21 700 ± 8 820	4.3 (3.5, 5.1)	<0.001
AUC(0,48 h) (ng ml^{-1} h)	5 440 ± 2 620	15 300 ± 4 790	3.2 (2.7, 3.8)	<0.001
CL/*F* kg^{-1} (l h^{-1} kg^{-1})	0.045 ± 0.02	0.011 ± 0.005	0.29 (0.24, 0.34)	<0.001
V/*F* (l)	36.7 ± 19.1	24.4 ± 6.2	0.80 (0.68, 0.91)	0.001
$t_{1/2}$ (h)	8.1 ± 2.9	23.7 ± 10.4	3.1 (2.6, 3.6)	<0.001

Data are expressed as mean ± SD and mean (95% confidence interval). C_{max} = maximum plasma concentration; AUC = area under the plasma concentration–time curve; CL/*F* kg^{-1} = weight-adjusted apparent oral clearance; *V*/*F* = apparent volume of distribution; $t_{1/2}$ = half-life.

parameters when it was given alone and in the presence of gemfibrozil were assessed with paired *t*-tests. Pharmacokinetic data, including relative changes, were compared between *CYP2C8 *1* homozygotes and *CYP2C8 *3* carriers, using independent Student's *t*-tests or Mann Whitney U tests (for time data). The relationship between gemfibrozil AUC(0,10 h) and the relative change in pioglitazone AUC(0,∞) was assessed by Pearson correlation coefficients. Statistical analyses were conducted using SPSS version 18.0 software. A *P* value <0.05 was considered statistically significant.

Results

One hundred forty-two subjects were prospectively genotyped for the *CYP2C8 *3* allele, and 34 subjects were started on study protocol. Four subjects withdrew after only one phase of the study due to personal reasons. Results are presented for the 30 subjects who completed both intensive pharmacokinetic study visits, i.e. pioglitazone alone and pioglitazone plus gemfibrozil. The study consisted of 21 women and nine men, mean age of 36 ± 11 years and mean weight of 72.7 ± 15.9 kg. Subjects had the following *CYP2C8* genotypes: **1/*1* ($n = 15$); **1/*3* ($n = 14$) and **3/*3* ($n = 1$). Baseline demographics did not differ significantly between *CYP2C8* genotype groups, and are shown in Table 1.

Pioglitazone pharmacokinetic parameters in the absence and presence of gemfibrozil in the entire study cohort ($n = 30$) are shown in Table 2. Gemfibrozil significantly increased mean pioglitazone AUC(0,∞) by 4.3-fold ($P < 0.001$) and there was substantial interindividual variability in the magnitude of this interaction (range, 1.8- to 12.1-fold). Gemfibrozil also significantly decreased pioglitazone weight-adjusted apparent oral clearance by approximately 70% ($P < 0.001$) and lengthened the mean $t_{1/2}$ of pioglitazone by 3-fold ($P < 0.001$). The mean C_{max} of pioglitazone did not significantly change following gemfibrozil administration. The median t_{max} of pioglitazone was 2.0 h in the absence and presence of gemfibrozil.

Pioglitazone plasma concentration–time curves in the absence and presence of gemfibrozil, by *CYP2C8* genotype group, are shown in Figure 1. Pioglitazone AUC(0,∞) in the absence and presence of gemfibrozil for each subject is shown in Figure 2. When pioglitazone was administered alone, mean AUC(0,∞) was 29.7% lower ($P = 0.01$) and mean weight-adjusted oral clearance was 64.7% higher ($P =$

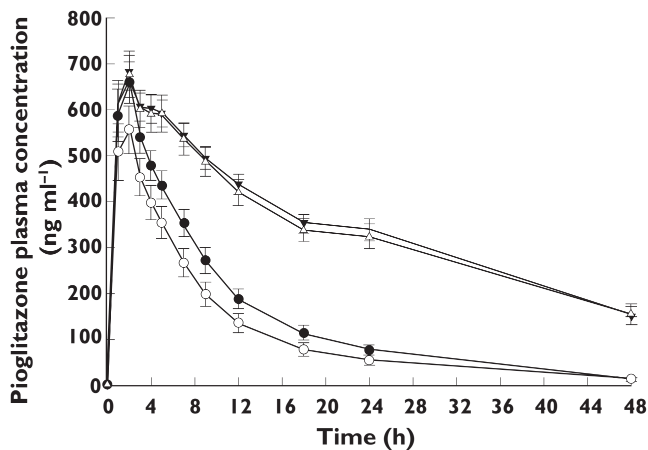

Figure 1

Pioglitazone plasma concentration–time curves by *CYP2C8* genotype group when pioglitazone was administered alone and in combination with gemfibrozil. *CYP2C8 *1/*1*, pioglitazone alone (–●–); *CYP2C8 *3* carriers, pioglitazone alone (–○–); *CYP2C8 *1/*1*, pioglitazone + gemfibrozil (–▼–); *CYP2C8 *3* carriers, pioglitazone + gemfibrozil (–△–). Data are shown as mean ± SEM

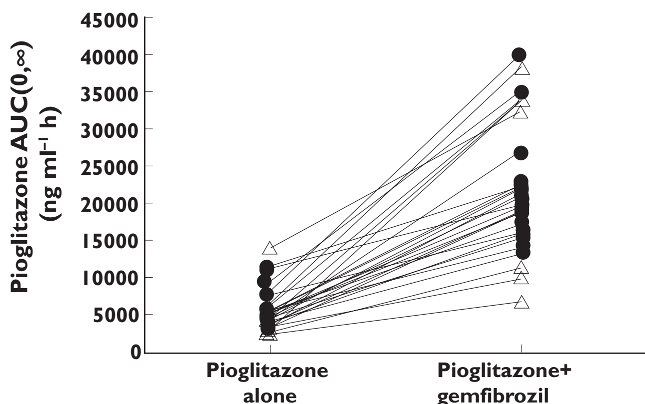

Figure 2

Pioglitazone AUC(0,∞) in the absence and presence of gemfibrozil for each participant. *CYP2C8 *1/*1* (–●–); *CYP2C8 *3* carriers (–△–)

0.002) in *CYP2C8 *3* carriers compared with *CYP2C8 *1* homozygotes (Table 3). In the presence of gemfibrozil, pioglitazone pharmacokinetic parameters did not differ significantly between *CYP2C8* genotype groups (Table 3). However, the mean relative change in pioglitazone pharmacokinetic parameters following gemfibrozil administration was significantly influenced by *CYP2C8* genotype (Table 3, Figure 3). Specifically, *CYP2C8*3* carriers had a mean 5.2-fold increase in pioglitazone AUC(0,∞) compared with a mean 3.3-fold increase in *CYP2C8 *1* homozygotes ($P = 0.02$) following gemfibrozil administration. The relative change in pioglitazone $t_{1/2}$ was also larger in *CYP2C8 *3* carriers compared with *CYP2C8 *1* homozygotes (3.3-fold *vs.* 2.9-fold), although this difference did not reach statistical significance. The subject with the largest relative increase in pioglitazone AUC(0,∞) (12.1-fold) had the *CYP2C8 *1/*3* genotype. There was one subject with the *CYP2C8 *3/*3* genotype in the study cohort and this subject experienced a 7.0-fold increase in pioglitazone AUC(0,∞). Of the 10 subjects with the largest relative increases in pioglitazone AUC(0,∞), eight subjects were *CYP2C8 *3* carriers.

Gemfibrozil and gemfibrozil 1-*O*-β-glucuronide pharmacokinetic parameters did not differ significantly between *CYP2C8* genotype groups (Table 4). Furthermore, gemfibrozil AUC(0,10 h) was not significantly correlated with the relative change in pioglitazone AUC(0,∞) in the entire study cohort ($r = 0.04$, $P = 0.83$), nor by *CYP2C8* genotype group (*CYP2C8 *1/*1*, $r = 0.03$, $P = 0.91$; *CYP2C8 *3* carriers, $r = 0.04$, $P = 0.90$).

Discussion

Previously, it has been shown that gemfibrozil increases pioglitazone plasma exposure in healthy volunteers, and interindividual variability exists in the magnitude of this interaction [5, 18]. We prospectively set out to determine if the *CYP2C8 *3* allele influences the extent of this inhibitory drug–drug interaction. Our primary finding was that the relative change in pioglitazone plasma exposure following gemfibrozil administration was significantly influenced by *CYP2C8* genotype. Specifically, the relative increase in pioglitazone plasma exposure was greater in *CYP2C8 *3* carriers compared with *CYP2C8 *1* homozygotes. These data suggest that a portion of the interindividual variability in the drug-drug interaction between gemfibrozil and pioglitazone may be explained by the *CYP2C8 *3* allele.

To date, most investigations of the impact of pharmacogenetics on inhibitory drug–drug interactions have been conducted in relation to CYP2D6 and CYP2C19 metabolizing enzymes, with a focus on extensive and poor metabolizers. In these cases, the magnitude of substrate inhibition tends to be greater in genetically-determined extensive metabolizers *vs.* poor metabolizers because inhibition cannot occur in individuals who lack the metabolizing enzyme [19–23]. However, less is known about the impact of *CYP2C8* polymorphisms or increased metabolic activity phenotypes (e.g. ultrarapid metabolizers) on inhibitory drug–drug interactions. To our knowledge, the finding that *CYP2C8* genotype significantly influences the magnitude of the interaction between gemfibrozil and pioglitazone has not been reported before.

When pioglitazone was administered alone, we found that its plasma exposure was significantly lower and weight-adjusted apparent oral clearance was significantly higher in carriers of the *CYP2C8 *3* allele as compared with *CYP2C8 *1* homozygotes. This finding is consistent with previous clinical reports of increased thiazolidinedione metabolism and decreased plasma exposure in carriers of

Table 3

Pioglitazone pharmacokinetic parameters by *CYP2C8* genotype group when pioglitazone was administered alone and in combination with gemfibrozil

Pharmacokinetic parameter	*CYP2C8 *1/*1* (*n* = 15)	*CYP2C8 *3* carriers (*n* = 15)	*P* value (between genotype groups)
C_{max} (ng ml^{-1})			
Pioglitazone	641 ± 171	575 ± 253	0.22
Pioglitazone + gemfibrozil	696 ± 164	660 ± 212	0.51
Mean relative change	1.1 (0.96, 1.3)	1.4 (0.88, 2.0)	0.28
P value (within genotype group)	0.30	0.21	
AUC(0,∞) (ng ml^{-1} h)			
Pioglitazone	6 770 ± 2 480	4 760 ± 2 900	0.01
Pioglitazone + gemfibrozil	21 100 ± 7 800	22 200 ± 9 970	0.99
Mean relative change	3.3 (2.7, 4.0)	5.2 (3.8, 6.7)	0.02
P value (within genotype group)	<0.001	<0.001	
AUC(0,48 h) (ng ml^{-1} h)			
Pioglitazone	6 340 ± 2 250	4 540 ± 2 730	0.01
Pioglitazone + gemfibrozil	15 800 ± 3 840	14 800 ± 5 670	0.39
Mean relative change	2.7 (2.2, 3.2)	3.7 (2.8, 4.7)	0.05
P value (within genotype group)	<0.001	<0.001	
CL/*F* kg^{-1} (l h^{-1} kg^{-1})			
Pioglitazone	0.034 ± 0.0097	0.056 ± 0.023	0.002
Pioglitazone + gemfibrozil	0.011 ± 0.0026	0.012 ± 0.0072	0.712
Mean relative change	0.34 (0.27, 0.42)	0.23 (0.17, 0.30)	0.02
P value (within genotype group)	<0.001	<0.001	
$t_{1/2}$ (h)			
Pioglitazone	8.2 ± 3.0	8.0 ± 3.0	0.85
Pioglitazone + gemfibrozil	21.7 ± 8.5	25.7 ± 12.0	0.30
Mean relative change	2.9 (2.1, 3.6)	3.3 (2.5, 4.1)	0.35
P value (within genotype group)	<0.001	<0.001	

Data are expressed as mean ± SD, or mean (95% confidence interval) for relative change data. C_{max} = maximum plasma concentration; AUC = area under the plasma concentration–time curve; CL/*F* kg^{-1} = weight-adjusted apparent oral clearance; $t_{1/2}$ = half-life.

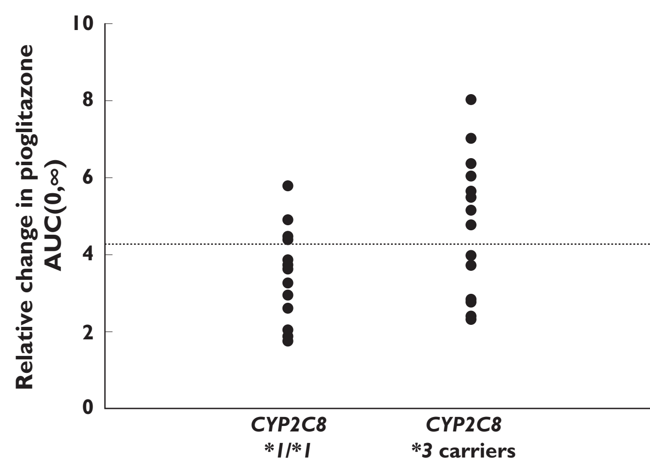

Figure 3

Relative change in pioglitazone AUC(0,∞) by *CYP2C8* genotype group. The dashed line represents the mean of the study cohort. *CYP2C8 *1/*1* (*n* = 15), *CYP2C8 *3* carriers (*n* = 15)

the *CYP2C8 *3* allele [31–33]. The observed magnitude of genotype effect was also similar to other clinical studies, with an approximate 25% to 30% lower pioglitazone plasma exposure in *CYP2C8 *3* carriers compared with wild-type homozygotes [31]. In terms of functional significance, there have been conflicting data regarding the effects of the *CYP2C8 *3* allele on substrate metabolism, with reports of increased metabolic activity, decreased metabolic activity, and substrate dependency. However, an *in vitro* study has recently shed more light on this topic [30]. Kaspera and colleagues found that recombinant *CYP2C8 *3* exhibited higher overall activity than *CYP2C8 *1* in the presence of cytochrome b5, a redox partner [30]. This finding is thought to be due to greater affinity of *CYP2C8 *3* for cytochrome b5 and cytochrome P450 reductase [30]. Taking recent *in vitro* and *in vivo* data together, it appears that *CYP2C8 *3* is associated with increased metabolism and decreased plasma concentrations of pioglitazone.

Our pharmacogenetic drug–drug interaction study found a significantly greater relative increase in pioglitazone plasma exposure in *CYP2C8 *3* carriers (5.2-fold) compared with *CYP2C8 *1* homozygotes (3.3-fold) following gemfibrozil administration. A few other studies have assessed the role of *CYP2C8* polymorphisms on the magnitude of CYP2C8-mediated thiazolidinedione drug–drug interactions. One study showed that trimethoprim, a weak competitive CYP2C8 inhibitor, increased the plasma exposure of pioglitazone by 42% in healthy volunteers [31].

Table 4

Gemfibrozil and gemfibrozil 1-*O*-β-glucuronide pharmacokinetic parameters by *CYP2C8* genotype group

Pharmacokinetic parameter	*CYP2C8 *1/*1* (n = 15)	*CYP2C8 *3* carriers (n = 15)	*P* value
Gemfibrozil			
C_{max} (μg ml^{-1})	24.9 ± 9.4	24.8 ± 9.5	0.96
AUC(0,10 h) (μg ml^{-1} h)	92.9 ± 37.3	94.9 ± 38.6	0.94
CL/F kg^{-1} (l h^{-1} kg^{-1})	0.1 ± 0.03	0.1 ± 0.03	0.88
$t_{1/2}$ (h)	2.0 ± 0.4	1.8 ± 0.3	0.20
t_{max} (h)	2.0 (2.0–3.0)	2.0 (2.0–4.0)	0.73
Gemfibrozil 1-*O*-β-glucuronide			
C_{max} (μg ml^{-1})	7.5 ± 6.6	6.8 ± 1.7	0.74
AUC(0,10 h) (μg ml^{-1} h)	30.7 ± 12.7	32.6 ± 8.6	0.45

Data are presented as mean ± SD or median (range).

However, the *CYP2C8 *3* allele did not influence the extent of the interaction. Along the same lines, another healthy volunteer study reported that fluvoxamine, a weak competitive CYP2C8 inhibitor, increased the plasma exposure of rosiglitazone by 21% and the effects were consistent across *CYP2C8* genotype groups [40]. The variable genetic findings between studies are not surprising given that different inhibitor-substrate combinations were tested in each of these scenarios. Gemfibrozil is one of the most potent *in vivo* CYP2C8 inhibitors, primarily due to mechanism-based inhibition of CYP2C8 by its 1-*O*-β-glucuronide metabolite [13, 15]. As such, gemfibrozil is classified by the Food and Drug Administration as a strong *in vivo* CYP2C8 inhibitor (i.e. ≥5-fold increase in plasma exposure of CYP2C8 substrates) [41, 42]. In contrast, trimethoprim and fluvoxamine are classified as weak competitive *in vivo* inhibitors of CYP2C8 (i.e., ≥1.25 but <2-fold increase in plasma exposure of CYP2C8 substrates) [41, 42]. It is reasonable to hypothesize that *CYP2C8 *3* carriers may be more susceptible to CYP2C8 inhibition by mechanism-based inhibitors, such as gemfibrozil 1-*O*-β-glucuronide, due to a greater amount of inactivating species produced as a result of the *CYP2C8* *3 allele. This hypothesis is consistent with data from Tornio and colleagues who showed that the interaction between gemfibrozil and repaglinide (a CYP2C8 substrate) was stronger in CYP2C8 **3* carriers than in non-carriers [14]. *In vitro* and *in vivo* studies are needed to elucidate further the impact of genetic polymorphisms on mechanism-based versus competitive inhibitory drug–drug interactions.

Our study highlights several important considerations regarding the impact of pharmacogenetics on the evaluation of inhibitory drug–drug interactions. As previously reviewed by Lee and colleagues, reports of drug–drug interaction data are often limited in scope because interindividual variability in the magnitude of the interaction is not fully explored nor explained [1]. As such, caution must be exerted when extrapolating mean pharmacokinetic drug interaction data to the clinical setting. Importantly, genetic subgroups may exist which are susceptible to differing magnitudes of the interaction. In the case of gemfibrozil-pioglitazone, we observed the mean relative change in pioglitazone plasma exposure to be 4.3-fold, with a range of 1.8-fold to 12.1-fold. Although pioglitazone plasma exposure in the presence of gemfibrozil did not differ significantly between *CYP2C8* genotype groups, the magnitude of change in pioglitazone pharmacokinetics was affected by *CYP2C8* genotype. With regards to clinical pharmacology and the drug development process, it would seem prudent to routinely interrogate *CYP2C8* polymorphisms when gemfibrozil is used as a CYP2C8 inhibitor, or when pioglitazone is used as a CYP2C8 probe drug, in order to characterize comprehensively the contribution of genetics to interindividual variability in the magnitude of potential drug–drug interactions.

There are limitations of our study that deserve to be acknowledged. First, based on previous findings of increased parent pioglitazone concentrations in the presence of gemfibrozil, we only measured parent pioglitazone concentrations in our study [5, 18]. Pioglitazone is metabolized to a number of different active metabolites, namely M-III, and M-IV [4]. A previous study found no significant differences in M-III or M-IV plasma exposure between *CYP2C8* genotype groups [31]. However, M-III : parent and M-IV : parent AUC ratios were significantly greater in carriers of *CYP2C8 *3* allele compared with wild-type homozygotes [31]. Second, only one subject with the *CYP2C8 *3/*3* genotype was present in our cohort. This subject experienced a 7.0-fold increase in pioglitazone plasma exposure, which was the third highest relative change among all subjects in the study. Additional studies are needed to determine if a *CYP2C8 *3* gene–dose effect exists during pioglitazone monotherapy and in the setting of inhibitory drug-drug interactions. Third, because we intentionally used a prospective *CYP2C8 *3* genotype enrichment design, we did not interrogate other polymorphisms in the *CYP2C8* gene

or other CYP metabolizing enzymes. Future consideration should be given to the effect of other polymorphic alleles (e.g., *CYP2C8 *4*, *CYP2C8* –271 C>A) or novel *CYP2C8* haplotypes on pioglitazone plasma concentrations and drug-drug interactions [28]. Along the same lines, future studies should evaluate the extent to which polymorphisms in UGT2B7, the enzyme that mediates the conversion of gemfibrozil to its 1-*O*-β-glucuronide metabolite, influence interindividual pharmacokinetic variability in gemfibrozil-mediated drug-drug interactions [43, 44]. Although not evaluated in our *CYP2C8* genotype-focused analysis, it is possible that polymorphic *UGT2B7* alleles are an additional source of variability in the pioglitazone-gemfibrozil interaction. However, it is unlikely that *UGT2B7* polymorphisms confounded our study results given that gemfibrozil and gemfibrozil 1-*O*-β-glucuronide plasma exposure did not differ significantly between *CYP2C8* genotype groups.

In summary, the polymorphic *CYP2C8 *3* allele influences pharmacokinetic variability in the magnitude of the drug–drug interaction between gemfibrozil and pioglitazone. Additional studies are needed to evaluate the impact of *CYP2C8* polymorphisms on other CYP2C8-mediated drug–drug interactions, particularly those involving mechanism-based inhibitors.

Competing Interests

There are no competing interests to declare.

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