Trimethoprim and the *CYP2C8*{3} allele have opposite effects on the pharmacokinetics of pioglitazone

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Running title:
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Abbreviations: AUC, area under concentration-time curve; PPAR-γ, peroxisome
proliferator-activated receptor gamma; CYP, Cytochrome P450; OATP, organic anion
transporting polypeptide; HPLC, high-performance liquid chromatography; HLM,
human liver microsomes.
Abstract:

We studied the effects of the CYP2C8 inhibitor trimethoprim and CYP2C8 genotype on the pharmacokinetics of the antidiabetic pioglitazone. In a randomized crossover study, 16 healthy volunteers with the CYP2C8*1/*1 (n=8), *1/*3 (n=5), or *3/*3 (n=3) genotype ingested 160 mg trimethoprim or placebo twice daily for 6 days. On day 3, they ingested 15 mg pioglitazone. In vitro, the effects of trimethoprim on pioglitazone were characterized. Trimethoprim raised the area under the plasma pioglitazone concentration-time curve (AUC\textsubscript{0-\infty}) by 42% ($p < 0.001$), and decreased the formation rates of pioglitazone metabolites M-IV and M-III ($p < 0.001$). During the placebo phase, the weight-adjusted AUC\textsubscript{0-\infty} of pioglitazone was 34% smaller in the CYP2C8*3/*3 and 26% smaller in the CYP2C8*1/*3 than in the CYP2C8*1/*1 group ($p < 0.05$). In vitro, trimethoprim inhibited M-IV formation (inhibition constant 38.2 µM), predicting the in vivo interaction. In conclusion, drug interactions and pharmacogenetics affecting the CYP2C8 enzyme may change the safety of pioglitazone.
The thiazolidinedione pioglitazone is a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist used in the treatment of type 2 diabetes. The oral bioavailability of pioglitazone is over 80%, and it is extensively (>97%) bound to plasma proteins (Eckland and Danhof, 2000). Pioglitazone is metabolized by hydroxylation and oxidation to several metabolites (Eckland and Danhof, 2000). The pharmacologically active M-IV and M-III (a secondary metabolite formed from M-IV) are the main metabolites found in human serum (Eckland and Danhof, 2000).

Unlike some earlier reports (Eckland and Danhof, 2000; Hanefeld, 2001), recent in vitro and in vivo studies strongly suggest that pioglitazone is metabolized mainly by cytochrome P450 (CYP) 2C8, and to a lesser extent by CYP3A4 (Jaakkola et al., 2005; Jaakkola et al., 2006b). Gemfibrozil, which is in vivo a strong inhibitor of CYP2C8 (Backman et al., 2002; Ogilvie et al., 2006) and of the hepatic uptake transporter organic anion transporting polypeptide 1B1 (OATP1B1) (Shitara et al., 2004), has increased the mean area under the plasma concentration-time curve (AUC) of pioglitazone by 3.2-fold (Jaakkola et al., 2005). On the other hand, itraconazole, a strong inhibitor of CYP3A4 (Back and Tjia, 1991; Olkkola et al., 1994), has had no effect on the pharmacokinetics of pioglitazone (Jaakkola et al., 2005), indicating that the role of CYP3A4 in the metabolism of pioglitazone in vivo is limited.

The CYP2C8 enzyme is genetically polymorphic, and certain variants causing functional effects on enzyme activity have been described (Dai et al., 2001). CYP2C8.3 (CYP2C8*3), with Arg139Lys and Lys399Arg amino acid substitutions, has shown a reduced turnover rate for paclitaxel, arachidonic acid, and amodiaquine in vitro (Dai et al., 2001; Parikh et al., 2007). In contrast to these in vitro findings, the CYP2C8*3 allele has been associated with increased clearance and decreased plasma concentrations of the oral antidiabetics repaglinide and rosiglitazone (Niemi et al., 2003c; Niemi et al., 2005; Kirchheiner et al., 2006). On the other hand, the clearance...
of the CYP2C8 substrate R-ibuprofen has been reduced in subjects carrying the CYP2C8*3 allele, compared to subjects with the CYP2C8*1/*1 reference genotype (García-Martín et al., 2004). Accordingly, the in vivo significance of the CYP2C8*3 allele has been somewhat controversial. The frequency of CYP2C8*3 in white subjects is about 10-20% and only about 2% in black subjects (Totah and Rettie, 2005).

The antimicrobial drug trimethoprim is a selective and moderately potent inhibitor of CYP2C8 in vitro (Wen et al., 2002). In vivo, trimethoprim has raised the AUC of the CYP2C8 substrates repaglinide and rosiglitazone by 61% and 37%, respectively (Niemi et al., 2004a; Niemi et al., 2004b). In the present study, we investigated the effects of trimethoprim, given 160 mg twice daily, and of the CYP2C8 genotype, on the pharmacokinetics of 15 mg pioglitazone in 8 non-carriers and 8 carriers of the CYP2C8*3 allele (Table 1). The predictability of the trimethoprim-pioglitazone interaction was also evaluated using in vitro pharmacokinetic studies.
Methods

Subjects. Sixteen healthy volunteers (8 men and 8 women; mean age 21 years, range 19-25 years; Table 1) participated in the study after giving written informed consent. They were recruited from a pool of more than 400 pharmacogenetically characterized subjects genotyped for CYP2C8 alleles. Genotyping for the CYP2C8*3 (c.416G>A, c.1196A>G) and CYP2C8*4 (c.792C>G) alleles was achieved by using Custom TaqMan® SNP genotyping assays on an Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Genotyping accuracy was validated against a previously described method (Niemi et al., 2003c). The participants were selected on the basis of the CYP2C8 genotype and allocated into three groups: CYP2C8*1/*1 (i.e., non-carriers of CYP2C8*3; n=8), CYP2C8*1/*3 (n=5), and CYP2C8*3/*3 (n=3). Only non-carriers of the CYP2C8*4 allele were recruited. None of the subjects had participated in our previous studies on the effects of the CYP2C8*3 allele on the pharmacokinetics of repaglinide (Niemi et al., 2003c; Niemi et al., 2005). The volunteers were ascertained to be healthy by medical history, physical examination, and routine laboratory tests before entering the study. None of the volunteers used continuous medication, e.g. oral contraceptives, or were smokers. The sample size was chosen so that a possible clinically significant pharmacokinetic drug interaction can be verified statistically without the use of an unnecessarily large group of healthy subjects. The number of subjects was estimated to be sufficient to detect a 25% change in the AUC0-∞ of pioglitazone with a power of 90% (alpha-level 5%).

Study design. The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District and the National Agency
for Medicines. A randomized, 2-phase crossover study with a washout period of 4 weeks was carried out. The volunteers received orally for six days 160 mg trimethoprim (Trimetin 160 mg tablet; Vitabalans, Hämeenlinna, Finland) or placebo (Yliopiston apteekki, Helsinki, Finland) twice daily at 8 AM and 8 PM with the exception of the evening dose on day 3, which was administered at 9 PM. On day 3, a single oral dose of 15 mg pioglitazone (one Actos 15 mg tablet; Takeda Europe, London, UK) was administered with 150 ml water at 9 AM, i.e. 1 hour after trimethoprim. The volunteers fasted overnight and had a warm standard meal 3 h and a light standard meal 7 and 11 h after pioglitazone intake.

**Sampling.** On the days of administration of pioglitazone, a forearm vein of each subject was cannulated with a plastic cannula and kept patent with an obturator. Timed blood samples were drawn prior to and 1, 2, 3, 4, 5, 7, 9, 12, 24, 48, 72, and 96 h after the administration of pioglitazone. The blood samples (10 ml each) were taken into tubes that contained ethylenediaminetetraacetic acid (EDTA). Plasma was separated within 30 min after blood sampling and stored at −70°C until analysis.

**Determination of drug concentrations.** Pioglitazone plasma and in vitro samples (0.5 ml) were spiked with 50 µl of internal standard (rosiglitazone, 3 µg/ml in 20% methanol) and 100 µl of perchloric acid (35%), vortex mixed and centrifuged. The supernatants were applied to the MCX solid phase extraction cartridges (Waters, Milford, MA, USA), which had been conditioned with 1 ml methanol and 1 ml of water. The cartridges were then washed with 1 ml 0.1 M hydrochloric acid and 1 ml of 70% methanol prior to elution with 1 ml of 2% ammonium hydroxide in methanol. The eluent was evaporated to dryness under nitrogen stream (50°C), after which the
residues were dissolved in 100 µl of acetonitrile-water (45:55 v/v) and transferred to autosampler vials.

The concentrations of pioglitazone and its metabolites M-IV and M-III were measured by use of PE SCIEX API 2000 liquid chromatography-tandem mass spectrometry system (Sciex division of MDS, Toronto, Canada) (Lin et al., 2003). Chromatography was performed on XTerra RP C18 column (3.9x100 mm; Waters, Milford, MA, USA) using gradient elution. The mobile phase consisted of 10 mM ammonium acetate (pH 9.5, adjusted with 25% ammonia solution) (A) and acetonitrile (B), and the flow rate was 400 µl/min. The mobile phase gradient comprised of 0 min at 5% B, 1 min to 20% B, 12 min to 60% B, 2 min at 100% B, and 5 min at 5% B, giving a total chromatographic run time of 20 min. The mass spectrometer was operated in the atmospheric pressure chemical ionization (APCI) with positive ion detection mode. The ion transitions monitored were m/z 357 to m/z 134 for pioglitazone, m/z 373 to m/z 150 for M-IV, m/z 371 to m/z 148 for M-III, and m/z 358 to m/z 135 for rosiglitazone. These transitions represent the product ions of the [M+H]+ ions of the parent pioglitazone, its two metabolites and the internal standard (Jaakkola et al., 2006a). The limit of quantification for pioglitazone, M-IV, and M-III was 0.1 ng/ml and the interday coefficients of variation (CV) were 5-10%, 9-14%, and 5-7% at relevant plasma concentrations, respectively. Trimethoprim did not interfere with the assay.

Plasma trimethoprim concentrations were measured by HPLC with ultraviolet detection (Weber et al., 1983; Svirbely and Pesce, 1987). The limit of quantification was 0.1 µg/ml, and the interday CVs were less than 5% at relevant concentrations.
Pharmacokinetics. The pharmacokinetics of pioglitazone were characterized by $C_{\text{max}}$, time to $C_{\text{max}}$, AUC$_{0-\infty}$, the dominant elimination half-life ($t_{1/2}$, the half-life of the phase contributing most to the area under the curve) and the terminal half-life ($t_{1/2,\text{terminal}}$). The pharmacokinetics of the metabolites M-IV and M-III were characterized by $C_{\text{max}}$, AUC$_{0-\infty}$ and $t_{1/2}$. Apparent formation rate constants were calculated for metabolites M-IV and M-III by individual modelling with a 1-compartment first-order formation model. In 15 of the 16 subjects, the plasma concentrations of pioglitazone declined biphasically during both study phases. In one subject (number 5), the concentration declined monophasically during both phases. Therefore, for the dominant elimination phase, the elimination rate constant ($k_e$) was determined by linear regression analysis using the first log-linear phase of the descending plasma concentration curve. For the slow elimination phase, a terminal elimination rate constant ($k_{e,\text{terminal}}$) was determined. The plasma concentrations of M-IV and M-III declined monophasically, and their elimination rate constants ($k_e$) were determined as above. The $t_{1/2}$ values were calculated from the equation $t_{1/2} = \ln2/k_e$. 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, by division of the last measured concentration by $k_{e,\text{terminal}}$ for pioglitazone, and $k_e$ for M-IV and M-III. The pharmacokinetics of trimethoprim was characterized by $C_{\text{max}}$ and AUC$_{0-13\,\text{h}}$ after the morning dose of trimethoprim on day 3. All the calculations were performed with MK-Model, version 5.0 (Biosoft, Cambridge, UK).

Statistical analysis. The results are expressed as mean values±SD in the text, tables and figures, unless otherwise indicated. The pharmacokinetic variables of pioglitazone and its metabolites between trimethoprim and placebo phases were compared with repeated measures ANOVA with the CYP2C8 genotype as a between-subjects factor.
Logarithmic transformation was applied for $C_{\text{max}}$ and AUC values. The differences between genotype groups were tested with 1-way ANOVA and post hoc comparisons with LSD. All the data were analyzed with SPSS for Windows, version 15.0.1 (SPSS Inc, Chicago, IL, USA). Differences were considered statistically significant at $p < 0.05$.

**In vitro study.** Pooled human liver microsomes (new catalogue number 452161, lot 20567), representing a pool from 18 individuals, and recombinant human CYP2C8 (new catalogue number 456252, lot 17461) and CYP3A4 (new catalogue number 456202, lot 18673) isoforms were purchased from BD Biosciences (Woburn, MA, USA). Human liver tissue had been collected in accordance with all pertinent regulations. The procedures of organ collection had been reviewed and accepted by the respective institutional Human Subjects Committee. Pioglitazone hydrochloride (Toronto Research Chemicals Inc., North York, Canada), pioglitazone metabolites M-IV and M-III (Synfine Research, Richmond Hill, Canada), trimethoprim and β-NADPH (Sigma-Aldrich, Steinheim, Germany) were used in this study. Other chemicals were obtained from Merck (Darmstadt, Germany).

The incubations were carried out in 0.1 M sodium phosphate buffer (pH 7.4), containing 5.0 mM MgCl$_2$, 0.3 mg/ml microsomal protein or 20 pmol/ml recombinant CYP, pioglitazone with or without trimethoprim, and 1.0 mM β-NADPH (Jaakkola et al., 2006b). The incubations were commenced by the addition of β-NADPH. Pioglitazone and trimethoprim were dissolved in methanol and the final methanol concentration was 1% in all incubations. The samples were incubated in duplicates at 37°C for 8 minutes, and the reactions were terminated by adding 100 µl perchloric acid (35%) to an aliquot (0.5 ml), and cooling on ice. The mean values of the duplicates were used in calculations.
The kinetics of pioglitazone M-IV metabolite formation were determined by incubating pioglitazone (0.375-50 µM) with HLM, recombinant CYP2C8, and CYP3A4. Using nonlinear regression, a model for either Michaelis-Menten kinetics (with or without substrate inhibition) or sigmoidal (Hill) kinetics was fitted to the data. The inhibition constant (Ki) for trimethoprim was determined by incubating pioglitazone (1.25-10 µM) with either HLM or recombinant CYP2C8 and trimethoprim (0-200 µM), followed by fitting different models of enzyme inhibition to the data. The nonlinear regression analysis was carried out with the program SigmaPlot for Windows, version 9.01 (Systat Software Inc., San Jose, CA, USA).

**In vitro – in vivo correlations.** To investigate the relationship between the in vitro inhibition of pioglitazone metabolism and the increase in the AUC of pioglitazone by trimethoprim (AUC\textsubscript{inhibited} / AUC\textsubscript{control}), the following equation was fitted to the observed individual data using regression analysis.

\[
\frac{AUC_{\text{inhibited}}}{AUC_{\text{control}}} = 1 + \frac{C_{\text{liver}} / C_{\text{plasma}} \text{ ratio} \cdot C_{\text{avg,trimethoprim}}}{K_i}
\]

The \(C_{\text{liver}}/C_{\text{plasma}}\) ratio is the liver(unbound, available to the enzyme)/plasma(total) concentration ratio of trimethoprim, \(K_i\) is the observed \(K_i\) of trimethoprim, and the \(C_{\text{avg,trimethoprim}}\) is the observed average (total) plasma concentration of trimethoprim, which was calculated as follows.

\[
C_{\text{avg,trimethoprim}} = \frac{AUC_{0-13\text{h}}}{13 \text{h}}
\]

where \(AUC_{0-13\text{h}}\) is the AUC of trimethoprim during the study day. The regression analysis was performed with SPSS for Windows, version 15.0.1.
Results

Effect of trimethoprim. Trimethoprim raised the mean AUC$_{0-\infty}$ of unchanged pioglitazone by 42% (range −19-128%; $p < 0.001$), compared with placebo (Table 2, Fig. 1). The dominant elimination $t_{1/2}$ of pioglitazone was prolonged from 3.9 h to 5.1 h ($p < 0.001$). However, the peak concentration in plasma ($C_{\text{max}}$), the time to $C_{\text{max}}$ (data not shown), or the terminal $t_{1/2}$ (data not shown) of pioglitazone was not changed significantly by trimethoprim.

The apparent formation rate constant ($k_f$) of the pioglitazone metabolite M-IV was 27% lower ($p < 0.001$) during the trimethoprim phase than during the placebo phase (Table 3). Trimethoprim also lowered the M-IV/pioglitazone AUC$_{0-\infty}$ ratio ($p < 0.001$). However, despite the reduced formation rate of the metabolite, the AUC$_{0-\infty}$ of M-IV was increased by 10% by trimethoprim ($p < 0.05$). The $k_f$ of the secondary pioglitazone metabolite M-III (formed from M-IV), and the M-III/pioglitazone AUC$_{0-\infty}$ ratio (mean±SD), were 0.13±0.044 (range 0.07-0.21) h$^{-1}$ and 0.74±0.12 (range 0.60-0.98) during the trimethoprim phase, and 0.17±0.080 (range 0.10-0.36; $p < 0.001$) h$^{-1}$ and 0.97±0.32 (range 0.5-1.7; $P=0.006$) during the placebo phase. No other significant changes were observed in the pharmacokinetic variables of M-III (data not shown).

Effect of CYP2C8 genotype. During the placebo phase, the subjects with the $CYP2C8^{*1/*1}$ or $*3/*3$ genotype had lower mean plasma concentrations of the parent pioglitazone than did the subjects with the $CYP2C8^{*1/*1}$ genotype (Fig. 2). The weight-adjusted AUC$_{0-\infty}$ of pioglitazone was 34% smaller in the $CYP2C8^{*3/*3}$ group (3.25±0.87 mg·h/l, $p < 0.05$), and 26% smaller in the $CYP2C8^{*1/*3}$ group (3.67±0.92 mg·h/l, $p < 0.05$) than in the $CYP2C8^{*1/*1}$ group (4.95±0.96 mg·h/l) (Table 2). In
line with this finding, the M-IV/pioglitazone and M-III/pioglitazone AUC<sub>0-∞</sub> ratios and apparent formation rate constants were higher in the variant groups than in the CYP2C8*1/*1 group (Table 3, Fig. 3). However, no statistically significant differences existed between the genotype groups in the AUC<sub>0-∞</sub> values of M-IV (Table 3) or M-III (data not shown), not even after weight-adjustment (data not shown).

During the trimethoprim phase, the differences in the pharmacokinetics of pioglitazone between the genotype groups were similar to those during the placebo phase or slightly smaller (Tables 2-3). There was no significant difference or even a tendency for differences in the extent of the trimethoprim-pioglitazone interaction between the three genotype groups (Table 2).

**Metabolism of pioglitazone in vitro.** The metabolism of pioglitazone to M-IV by pooled human liver microsomes (HLM) was adequately described by a Michaelis-Menten equation with a Michaelis-Menten constant (K<sub>m</sub>) of 9.8 µM and maximum velocity (V<sub>max</sub>) of 640 pmol/min/mg protein (data not shown). The formation of M-IV by recombinant CYP2C8 was best described by a model for Michaelis-Menten kinetics with substrate inhibition, whereas the formation by CYP3A4 was best described by sigmoidal (Hill) kinetics (Fig. 4A). The formation rate of the M-IV metabolite was 5-10 times higher by CYP2C8 than CYP3A4 at clinically relevant pioglitazone concentrations (< 2 µM). Trimethoprim concentration-dependently inhibited M-IV formation in human liver microsomes and recombinant CYP2C8, and the inhibitory effect was best described by the competitive inhibition model, with a mean (±standard error) inhibition constant (K<sub>i</sub>) of 38.2±3.9 µM and 34.1±4.0 µM, respectively (Fig. 4B).
Pharmacokinetics of trimethoprim and in vitro – in vivo correlations. The AUC$_{0-13h}$ and $C_{\text{max}}$ of trimethoprim on the day of pioglitazone administration were 28.6±9.0 (range 7.8-42) mg·h/l and 3.1±0.94 (range 1.2-4.7) µg/ml, respectively. One of the male subjects in the $CYP2C8^*1/*1$ group had exceptionally low trimethoprim concentrations (Table 1). The AUC$_{0-13h}$ of trimethoprim correlated significantly with the change in pioglitazone AUC$_{0-\infty}$ between the phases (pioglitazone AUC$_{\text{inhibited}}$ – pioglitazone AUC$_{\text{control}}$) (Pearson $r^2 = 0.577$, $p < 0.001$).

The extent of trimethoprim-pioglitazone interaction (pioglitazone AUC$_{\text{inhibited}}$/pioglitazone AUC$_{\text{control}}$) could be predicted reasonably accurately in two thirds of the subjects on the basis of the in vitro $K_i$ of trimethoprim and its average plasma concentration using a competitive inhibition model with a $C_{\text{liver}}$/C$_{\text{plasma}}$ ratio of 2.35 for trimethoprim (Fig. 5). The equation obtained using regression analysis explained 24% of the variability in the extent of interaction with a mean prediction error of −6%±22%. For 11 of the 16 subjects, the predictions were within ±20% of the observed extent of interaction. Of the five subjects outside the ±20% range, two had the $CYP2C8^*3/*3$ genotype, and two had the $CYP2C8^*1/*3$ genotype.
Discussion

In the current study, trimethoprim moderately and concentration-dependently increased the AUC\(_{0-\infty}\) of pioglitazone and lowered the ratio of the main primary metabolite M-IV to pioglitazone in plasma, indicating that trimethoprim inhibited the CYP2C8-mediated metabolism of pioglitazone. Trimethoprim also prolonged the dominant elimination \(t_{1/2}\) of pioglitazone. However, the terminal \(t_{65}\), which seems to reflect a release of pioglitazone from peripheral tissues and is insensitive also to the effect of induction (Jaakkola et al., 2006a), was unaffected by trimethoprim.

Compared to individuals with the CYP2C8*1/*1 genotype, those homozygous or heterozygous for the CYP2C8*3 variant allele had lower plasma concentrations of pioglitazone and a higher rate of metabolite formation. Both the weight adjusted AUC\(_{0-\infty}\) and the M-IV/pioglitazone and M-III/pioglitazone AUC\(_{0-\infty}\) ratios showed a gene-dose effect, with the heterozygous CYP2C8*3 allele carriers in between the homozygous carriers and the non-carrier group. The extent of the trimethoprim-pioglitazone interaction, which was independent of the CYP2C8 genotype, could be predicted using the \(K_i\) value of trimethoprim for CYP2C8 and plasma trimethoprim concentrations.

The adverse effects of the thiazolidinediones pioglitazone and rosiglitazone include fluid retention and peripheral edema, and seem to be dose (concentration) dependent (Hanefeld and Belcher, 2001; Kermani and Garg, 2003). Rosiglitazone has also been suspected of increasing the risk of cardiovascular death (Nissen and Wolski, 2007). Although the interaction of trimethoprim with pioglitazone was moderate, the increase in the AUC of pioglitazone was more than 2-fold in some subjects, and also the AUC of the active metabolite M-IV was increased by trimethoprim. Accordingly, it is possible that a concomitant administration of trimethoprim or other inhibitors of CYP2C8 with pioglitazone could lead to an
increased risk of concentration-dependent adverse effects. The pharmacokinetic profile of pioglitazone in patients with type 2 diabetes is similar to that in healthy volunteers (Eckland and Danhof, 2000). Thus, it is likely that also the interaction observed in the present study is similar in patients with type II diabetes. The opposite effect of the \( CYP2C8*3 \) allele is of the same magnitude as the trimethoprim-pioglitazone interaction. Most probably, the genotype effect is maintained also during long-term treatment with pioglitazone, as is the case with rosiglitazone, on which the \( CYP2C8*3 \) allele was recently found by Kirchheiner \textit{et al.} to have a similar effect (Kirchheiner \textit{et al.}, 2006). The efficacy of pioglitazone could be reduced, and the dose requirement be increased, in the \( CYP2C8*3 \) variant allele carriers, but this was not investigated in the current study. Furthermore, it is reasonable to assume that the risk of concentration-dependent adverse effects of pioglitazone (and rosiglitazone) could be dependent on the \( CYP2C8 \) genotype, but further studies are needed to test this hypothesis.

There has been a lot of controversy concerning the effect of the \( CYP2C8*3 \) allele on the disposition of different drugs, and no studies concerning its effect on the pharmacokinetics of pioglitazone have been published previously. In in vitro studies, CYP2C8.3 has had a reduced activity to metabolize paclitaxel, arachidonic acid and amodiaquine (Dai \textit{et al.}, 2001; Bahadur \textit{et al.}, 2002; Parikh \textit{et al.}, 2007). Surprisingly, in first human studies, carriers of the \( CYP2C8*3 \) allele were found to have lower plasma concentrations of repaglinide than the control group homozygous for the \( CYP2C8*1 \) allele (Niemi \textit{et al.}, 2003c; Niemi \textit{et al.}, 2005). Also with rosiglitazone, subjects homozygous for \( CYP2C8*3 \) have had an increased clearance compared to subjects homozygous for the \( CYP2C8*1 \) allele (Kirchheiner \textit{et al.}, 2006). On the other hand, some studies have found no significant effects of the \( CYP2C8*3 \) genotypes on the pharmacokinetics of repaglinide, rosiglitazone, or
paclitaxel (Henningsson et al., 2005; Bidstrup et al., 2006; Pedersen et al., 2006). Moreover, the \textit{CYP2C8*3} allele has been associated with a reduced clearance and prolonged \( t_{1/2} \) of both enantiomers of ibuprofen (García-Martín et al., 2004). Due to strong linkage disequilibrium, the \textit{CYP2C9*2} allele exists commonly in the same haplotype with the \textit{CYP2C8*3} allele (Yasar et al., 2002), which could affect these findings, since ibuprofen is metabolized by both of these CYP enzymes (Hamman et al., 1997). Thus, the result of the current study, that pioglitazone concentrations are lower in carriers of \textit{CYP2C8*3} allele than in non-carriers, together with the previous in vivo results with repaglinide and rosiglitazone (Niemi et al., 2003c; Niemi et al., 2005; Kirchheiner et al., 2006), suggest that the \textit{CYP2C8*3} allele is often associated with an increased CYP2C8 activity phenotype. The extent of the interaction between trimethoprim and pioglitazone, however, did not appear to depend on the CYP2C8 genotype.

The earlier information on the contribution of different CYP enzymes to the metabolism of pioglitazone has been discrepant. Thus, the European product information of Actos stated that the metabolism of pioglitazone occurs predominantly via CYP3A4 and CYP2C9 (Lääketietokeskus, 2007), whereas the U.S. label stated that the major CYP isoforms are CYP2C8 and CYP3A4 (FDA). According to our recent in vitro study, pioglitazone (at 1 \( \mu \)M concentration) is metabolized mainly (60-90\%) by CYP2C8, and to a lesser extent (10-40\%) by CYP3A4, while other CYP forms play a negligible role (Jaakkola et al., 2006b). However, previous findings that itraconazole has no effect on the pharmacokinetics of pioglitazone in humans (Jaakkola et al., 2005) suggest that also CYP3A4 has a negligible role in the metabolism of pioglitazone in vivo. The present in vitro results can provide an explanation for these apparent discrepancies between the in vitro and in vivo findings, as the role of CYP3A4 in the metabolism of pioglitazone became very small (< 10\%).
of the activity of CYP2C8; Fig. 4A) at clinically relevant pioglitazone concentrations
of < 2 µM (< 720 ng/ml) (Eckland and Danhof, 2000), due to the sigmoidal kinetics
of M-IV formation by CYP3A4. Sigmoidal kinetics is a common feature of CYP3A4
mediated reactions and a possible cause of overestimation of the role of the CYP
form, if too high substrate concentrations are used in vitro. It should be noted that the
free fraction of pioglitazone in plasma is less than 3%, and therefore its unbound
concentrations at the enzyme site (in the liver) are probably much smaller than its
total plasma concentrations. This example emphasizes the need of using relevant
concentrations when in vitro to in vivo correlations are made.

Repaglinide and rosiglitazone have been recommended to be used as in
vivo probes for CYP2C8-mediated metabolism (Huang et al., 2007). Repaglinide is a
sensitive probe drug, but unlike pioglitazone and rosiglitazone it easily causes
hypoglycemia. Moreover, repaglinide is also a substrate of OATP1B1, and its
pharmacokinetics are affected by the SLCO1B1 genotype (Niemi et al., 2005), unlike
those of pioglitazone (Kallikoski et al., 2007). Gemfibrozil has raised the AUC of
rosiglitazone and pioglitazone by 2.3-fold and 3.2-fold, respectively (Niemi et al.,
2003a; Jaakkola et al., 2005). In the present study, trimethoprim increased the AUC of
pioglitazone slightly more (by 42%) than it has raised the AUC of rosiglitazone (by
37%), suggesting that the role of CYP2C8 is slightly greater in the metabolism of
pioglitazone than in the metabolism of rosiglitazone. In addition, pioglitazone does
not inhibit CYP2C8 in vivo (Kajosaari et al., 2006). Taken together, pioglitazone
could serve as a more sensitive in vivo probe for CYP2C8 than rosiglitazone, while
having a better single dose safety profile than repaglinide.

In human liver microsomes, trimethoprim inhibited the formation of M-
IV from pioglitazone with a $K_i$ of 38 µM, which is comparable to the $K_i$ value (32
µM) of trimethoprim for the CYP2C8 model reaction, paclitaxel 6α-hydroxylation
(Wen et al., 2002). If this data were directly extrapolated from in vitro to in vivo, assuming similar trimethoprim concentrations in plasma and at the enzyme site in the liver, the actual interaction between trimethoprim and pioglitazone would be somewhat underestimated. The liver/plasma ratio of trimethoprim in rhesus monkeys has been about 6.5 (Craig and Kunin, 1973), suggesting that trimethoprim may be concentrated into the liver also in humans. In fact, incorporating the observed \( k_i \), average total plasma trimethoprim concentration and degree of interaction into a predictive model, the liver/plasma concentration ratio of trimethoprim was estimated to average 2.4 in the current study. Including the assumption that only 80% of pioglitazone is metabolized by CYP2C8, the \( C_{\text{liver}}/C_{\text{plasma}} \) ratio of trimethoprim was calculated to be 3.4 (data not shown). Thus, the (unbound) trimethoprim concentration available to CYP2C8 in the liver seems to be several-fold higher than the total plasma trimethoprim concentration. Furthermore, it should be noted that the unbound fraction of trimethoprim in plasma is about 55%.

To conclude, trimethoprim moderately increases the plasma concentrations of pioglitazone by inhibiting its CYP2C8-catalyzed biotransformation. The \( \text{CYP2C8}^{*3} \) allele is associated with reduced pioglitazone plasma concentrations and an increased activity of its CYP2C8-mediated metabolism. Thus, both drug interactions and genetic factors can change the pharmacokinetics of pioglitazone, potentially affecting its efficacy and safety.
DMD #18010

References


Footnotes:

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Legends to figures

Figure 1
Mean±SD plasma concentrations of pioglitazone, and its metabolites M-IV and M-III in 16 healthy volunteers after a single oral dose of 15 mg pioglitazone on day 3 of a 6-day treatment with placebo or 160 mg trimethoprim twice daily. Open circles, pioglitazone during placebo; solid circles, pioglitazone during trimethoprim. Inset depicts the same data on a semi-logarithmic scale.

Figure 2
Mean±SD weight-adjusted plasma concentrations of pioglitazone in different CYP2C8 genotype groups after a single oral dose of 15 mg pioglitazone during the placebo phase. Open circles, CYP2C8*1/*1 genotype (n=8); solid squares, CYP2C8*1/*3 genotype (n=5); solid triangles, CYP2C8*3/*3 genotype (n=3). Inset depicts the data on a semi-logarithmic scale. For clarity, some error bars have been omitted.

Figure 3
Box plot diagram showing the weight-adjusted pioglitazone AUC$_{0-\infty}$, the M-IV/pioglitazone AUC$_{0-\infty}$ ratio, and the M-III/pioglitazone AUC$_{0-\infty}$ ratio in subjects with the CYP2C8*1/*1 (n=8), *1/*3 (n=5), and *3/*3 (n=3) genotypes during the placebo phase. Boxes represent the range between the 25th and 75th percentiles. The horizontal lines within the boxes are medians, and the whiskers represent the range except for the outliers (open circle).
Figure 4

A, Formation of the main primary metabolite M-IV from pioglitazone by recombinant human CYP2C8 and CYP3A4 enzymes in vitro, and the corresponding kinetic parameters (±standard error). The fitted lines represent the Michaelis-Menten model with substrate inhibition for CYP2C8, and the sigmoidal kinetic model for CYP3A4. \( K_m \), Michaelis-Menten constant; \( V_{max} \), maximum reaction velocity; \( K_i \), inhibition constant for substrate inhibition; \( n \), Hill coefficient; Closed circles, CYP2C8; Open circles, CYP3A4. B, Michaelis-Menten plots and the \( K_i \) (±standard error) for inhibition of M-IV formation by trimethoprim in human liver microsomes. All data points are means±SD from duplicate determinations.

Figure 5

Relationship between trimethoprim average concentration and the fold change in pioglitazone AUC (AUC_{inhibited}/AUC_{control}) in 16 healthy volunteers. Open circles, CYP2C8*1/*1 genotype; solid squares, CYP2C8*1/*3 genotype; solid triangles, CYP2C8*3/*3 genotype. The fitted lines represent the \( C_{liver}/C_{plasma} \) ratio of trimethoprim (2.35±0.39) and its 95% confidence interval (dotted lines) derived by fitting the equation depicted in the figure to the data. \( C_{trimethoprim,avg} \), trimethoprim average concentration derived from trimethoprim AUC_{0-13 h}. 
### Table 1

**Characteristics of subjects.**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>CYP2C8 genotype</th>
<th>Trimethoprim C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>Trimethoprim AUC&lt;sub&gt;0-13 h&lt;/sub&gt; (mg·h/l)</th>
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<td>3.3±1.2</td>
<td>30.3±10.4</td>
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</table>

BMI, body mass index; C<sub>max</sub>, peak plasma concentration; AUC<sub>0-13 h</sub>, area under the plasma concentration-time curve from time 0 to 13 h after the morning dose of trimethoprim on day 3.
Table 2

Pharmacokinetic variables of pioglitazone after a single oral dose of 15 mg pioglitazone on day 3 of a 6-day treatment with placebo or 160 mg trimethoprim twice daily in subjects with the CYP2C8*1/*1 genotype (n = 8), CYP2C8*1/*3 genotype (n = 5), and CYP2C8*3/*3 genotype (n = 3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo phase (control)</th>
<th>Trimethoprim phase</th>
<th>Trimethoprim phase percent of control, mean (range)</th>
<th>p value between phases</th>
<th>p value between genotypes</th>
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<tbody>
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<td>C_{max} (ng/ml)</td>
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<td></td>
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<td></td>
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<tr>
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<td>659±173</td>
<td>110 (65-183)</td>
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<tr>
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<td>566±123</td>
<td>707±159</td>
<td>125 (93-233)</td>
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<td>*3/*3</td>
<td>511±213</td>
<td>593±169</td>
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<tr>
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<td>116 (65-233)</td>
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<td>0.476</td>
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<tr>
<td>*1/*1</td>
<td>4.5±0.6</td>
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<td>140 (111-159)</td>
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<td>*3/*3</td>
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<tr>
<td>mean</td>
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<td>C_{max,adj} (ng/ml)</td>
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<td>542±113</td>
<td>667±97</td>
<td>123 (93-233)</td>
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<tr>
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<tr>
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<td>622±100</td>
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<td>0.021</td>
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<td>AUC_{0-∞,adj} (mg h/l)</td>
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<tr>
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<tr>
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<tr>
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<td>4.23±1.15</td>
<td>5.92±1.56</td>
<td>140 (81-228)</td>
<td>&lt;0.001</td>
<td>0.017</td>
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</table>

Values are mean±SD, unless otherwise indicated; C_{max}, peak plasma concentration; t_{1/2}, dominant elimination half-life; AUC_{0-∞}, area under the plasma concentration-time curve from time 0 to infinity; C_{max,adj}, peak plasma concentration adjusted for 70 kg body weight; AUC_{0-∞,adj}, area under the plasma concentration-time curve from time 0 to infinity adjusted for 70 kg body weight.

\(^a\) p value from repeated measures ANOVA with genotype as between subjects factor.

\(*\) p < 0.05 versus *1/*1; † p < 0.05 versus *1/*3.
Table 3

Pharmacokinetic variables of the pioglitazone main primary metabolite M-IV after a single oral dose of 15 mg pioglitazone on day 3 of a 6-day treatment with placebo or 160 mg trimethoprim twice daily in subjects with the *CYP2C8*1/*1 genotype (n = 8), *CYP2C8*1/*3 genotype (n = 5), and *CYP2C8*3/*3 genotype (n = 3).

<table>
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<tr>
<th>Variable</th>
<th>Placebo phase (control)</th>
<th>Trimethoprim phase percent of control, mean (range)</th>
<th>p value between phasesa</th>
<th>p value between genotypesa</th>
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<td></td>
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<tr>
<td>1/*1</td>
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<tr>
<td>1/*3</td>
<td>0.23±0.086**</td>
<td>0.12±0.045</td>
<td>51 (35-71)</td>
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<tr>
<td>3/*3</td>
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<td>0.11±0.042</td>
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</tr>
<tr>
<td>mean</td>
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<td>0.10±0.040</td>
<td>61 (35-124)</td>
<td>&lt;0.001 0.057</td>
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<td><em>C_max (ng/ml)</em></td>
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</tr>
<tr>
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<td>300±47.0</td>
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Values are mean±SD, unless otherwise indicated; *k_f*, apparent formation rate constant; *C_max*, peak plasma concentration; *t_1/2*, elimination half-life; *AUC_0-∞*, area under the plasma concentration-time curve from time 0 to infinity.

a p value from repeated measures ANOVA with genotype as between subjects factor.

* p < 0.05 versus 1/*1, ** p < 0.01 versus 1/*1, *** p < 0.001 versus 1/*1
† p < 0.01 versus 1/*3.
Fig. 3

- **Pioglitazone AUC (mg·h/l)**
  - CYP2C8 genotype
  - *1/*1: 4.0, *1/*3: 2.0, *3/*3: 1.0
  - P = 0.045

- **M-IV to pioglitazone AUC ratio**
  - CYP2C8 genotype
  - *1/*1: 3.0, *1/*3: 2.0, *3/*3: 1.0
  - P = 0.031
  - *1/*1: 2.0, *1/*3: 1.0, *3/*3: 0.5
  - P = 0.008

- **M-III to pioglitazone AUC ratio**
  - CYP2C8 genotype
  - *1/*1: 1.5, *1/*3: 1.0, *3/*3: 0.5
  - P = 0.037
Fig. 4

A

M-IV formation rate (pmol/min/pmol)

0 10 20 30 40 50 60

Pioglitazone (μM)

- CYP2C8
  $K_m = 10.2 \pm 2.2 \, \mu M$
  $V_{max} = 9.2 \pm 1.2 \, \text{pmol/min/pmol}$
  $K_i = 42.3 \pm 11.4 \, \mu M$

- CYP3A4
  $K_m = 13.2 \pm 2.6 \, \mu M$
  $V_{max} = 3.9 \pm 0.4 \, \text{pmol/min/pmol}$
  $n = 1.6 \pm 0.3$

B

M-IV formation rate (pmol/min/mg)

0 2 4 6 8 10 12

Pioglitazone (μM)

- 0 μM trimethoprim
- 25 μM
- 50 μM
- 100 μM
- 200 μM

$K_i = 38.2 \pm 3.9 \, \mu M$
\[
\frac{AUC_{\text{inhibited}}}{AUC_{\text{control}}} = 1 + \frac{C_{\text{liver}}/C_{\text{plasma}} \cdot \text{ratio} \cdot C_{\text{avg,trimethoprim}}}{K_i}
\]

\[C_{\text{liver}}/C_{\text{plasma}} = 2.35 (1.52 - 3.19 \text{ 95% CI})\]

\[K_i = 38.2 \mu M\]