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

Aleksi Tornio, Mikko Niemi, Pertti J. Neuvonen and Janne T. Backman

Department of Clinical Pharmacology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

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Running title:

Effects of trimethoprim and the *CYP2C8*3* allele on pioglitazone

Address correspondence to:

Janne T. Backman, MD

Department of Clinical Pharmacology, University of Helsinki

Haartmaninkatu 4

FIN-00290 Helsinki, 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 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 ($AUC_{0-\infty}$) 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 $AUC_{0-\infty}$ 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.

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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 $AUC_{0-\infty}$ 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 C_{18} 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.

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Pharmacokinetics. The pharmacokinetics of pioglitazone were characterized by C_{max}, time to C_{max}, $AUC_{0-\infty}$, the dominant elimination half-life ($t_{½}$, the half-life of the phase contributing most to the area under the curve) and the terminal half-life ($t_{½,terminal}$). The pharmacokinetics of the metabolites M-IV and M-III were characterized by C_{max}, $AUC_{0-\infty}$ and $t_{½}$. 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 ($k_{e,terminal}$) 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 $t_{½}$ values were calculated from the equation $t_{½} = \ln 2/k_e$. The AUC values were calculated by use of the linear trapezoidal rule for the rising phase of the plasma concentration-time curve and the loglinear trapezoidal rule for the descending phase, with extrapolation to infinity, by division of the last measured concentration by $k_{e,terminal}$ for pioglitazone, and k_e for M-IV and M-III. The pharmacokinetics of trimethoprim was characterized by C_{max} and $AUC_{0-13\ h}$ 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_{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.

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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 (K_i) 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 ($AUC_{inhibited}$ / $AUC_{control}$), the following equation was fitted to the observed individual data using regression analysis.

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

The C_{liver}/C_{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 $C_{avg,trimethoprim}$ is the observed average (total) plasma concentration of trimethoprim, which was calculated as follows.

$$C_{avg,trimethoprim} = \frac{AUC_{0-13h}}{13\,h},$$

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

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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 $t_{½}$ of pioglitazone was prolonged from 3.9 h to 5.1 h ($p < 0.001$). However, the peak concentration in plasma (C_{max}), the time to C_{max} (data not shown), or the terminal $t_{½}$ (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/*3* 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

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line with this finding, the M-IV/pioglitazone and M-III/pioglitazone $AUC_{0-\infty}$ 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 $AUC_{0-\infty}$ 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_m) of 9.8 μM and maximum velocity (V_{max}) 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_i) of 38.2±3.9 μM and 34.1±4.0 μM, respectively (Fig. 4B).

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Pharmacokinetics of trimethoprim and in vitro – in vivo correlations. The $AUC_{0\text{-}13h}$ and C_{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 $AUC_{0\text{-}13h}$ of trimethoprim correlated significantly with the change in pioglitazone $AUC_{0\text{-}\infty}$ between the phases (pioglitazone $AUC_{inhibited}$ − pioglitazone $AUC_{control}$) (Pearson $r^2 = 0.577$, $p < 0.001$).

The extent of trimethoprim-pioglitazone interaction (pioglitazone $AUC_{inhibited}$ / pioglitazone $AUC_{control}$) 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_{liver}/C_{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.

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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 $t_{½}$ of pioglitazone. However, the terminal $t_{½}$, 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 *et al.* to have a similar effect (Kirchheiner 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 et al., 2001; Bahadur et al., 2002; Parikh 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 et al., 2003c; Niemi 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 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 *CYP2C8*3* allele has been associated with a reduced clearance and prolonged $t_{½}$ of both enantiomers of ibuprofen (García-Martín et al., 2004). Due to strong linkage disequilibrium, the *CYP2C9*2* allele exists commonly in the same haplotype with the *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 *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 *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 µM 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%

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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 (Kalliokoski 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_{liver}/C_{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 *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.

References

Back DJ and Tjia JF (1991) Comparative effects of the antimycotic drugs ketoconazole, fluconazole, itraconazole and terbinafine on the metabolism of cyclosporin by human liver microsomes. *Br J Clin Pharmacol* **32:**624-626.

Backman JT, Kyrklund C, Neuvonen M and Neuvonen PJ (2002) Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* **72:**685-691.

Bahadur N, Leathart JB, Mutch E, Steimel-Crespi D, Dunn SA, Gilissen R, Houdt JV, Hendrickx J, Mannens G, Bohets H, Williams FM, Armstrong M, Crespi CL and Daly AK (2002) CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. *Biochem Pharmacol* **64:**1579-1589.

Bidstrup TB, Damkier P, Olsen AK, Ekblom M, Karlsson A and Brøsen K (2006) The impact of CYP2C8 polymorphism and grapefruit juice on the pharmacokinetics of repaglinide. *Br J Clin Pharmacol* **61:**49-57.

Craig WA and Kunin CM (1973) Distribution of trimethoprim-sulfamethoxazole in tissues of rhesus monkeys. *J Infect Dis* **128:**Suppl:575-579 p.

Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI and Goldstein JA (2001) Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* **11:**597-607.

Eckland DA and Danhof M (2000) Clinical pharmacokinetics of pioglitazone. *Exp Clin Endocrinol Diabetes* **108(Suppl 2):**234-242.

FDA (Food and Drug Administration): Actos prescribing information. Available from: URL: www.fda.gov/cder/foi/label/1999/21073lbl.pdf. Accessed Jul 12, 2007.

García-Martín E, Martínez C, Tabarés B, Frías J and Agúndez JA (2004) Interindividual variability in ibuprofen pharmacokinetics is related to interaction of cytochrome P450 2C8 and 2C9 amino acid polymorphisms. *Clin Pharmacol Ther* **76:**119-127.

Hamman MA, Thompson GA and Hall SD (1997) Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochem Pharmacol* **54:**33-41.

Hanefeld M (2001) Pharmacokinetics and clinical efficacy of pioglitazone. *Int J Clin Pract Suppl***:**19-25.

Hanefeld M and Belcher G (2001) Safety profile of pioglitazone. *Int J Clin Pract Suppl***:**27-31.

Henningsson A, Marsh S, Loos WJ, Karlsson MO, Garsa A, Mross K, Mielke S, Vigano L, Locatelli A, Verweij J, Sparreboom A and McLeod HL (2005) Association of CYP2C8, CYP3A4, CYP3A5, and ABCB1 polymorphisms with the pharmacokinetics of paclitaxel. *Clin Cancer Res* **11:**8097-8104.

Huang SM, Temple R, Throckmorton DC and Lesko LJ (2007) Drug interaction studies: study design, data analysis, and implications for dosing and labeling. *Clin Pharmacol Ther* **81:**298-304.

Jaakkola T, Backman JT, Neuvonen M, Laitila J and Neuvonen PJ (2006a) Effect of rifampicin on the pharmacokinetics of pioglitazone. *Br J Clin Pharmacol* **61:**70-78.

Jaakkola T, Backman JT, Neuvonen M and Neuvonen PJ (2005) Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics of pioglitazone. *Clin Pharmacol Ther* **77:**404-414.

Jaakkola T, Laitila J, Neuvonen PJ and Backman JT (2006b) Pioglitazone is metabolised by CYP2C8 and CYP3A4 in vitro: potential for interactions with CYP2C8 inhibitors. *Basic Clin Pharmacol Toxicol* **99:**44-51.

Kajosaari LI, Jaakkola T, Neuvonen PJ and Backman JT (2006) Pioglitazone, an in vitro inhibitor of CYP2C8 and CYP3A4, does not increase the plasma concentrations of the CYP2C8 and CYP3A4 substrate repaglinide. *Eur J Clin Pharmacol* **62:**217-223.

Kalliokoski A, Neuvonen M, Neuvonen PJ and Niemi M (2007) No significant effect of SLCO1B1 polymorphism on the pharmacokinetics of rosiglitazone and pioglitazone. *Br J Clin Pharmacol:* Epub 2007 Jul 17 doi:10.1111/j.1365-2125.2007.02986.x.

Kermani A and Garg A (2003) Thiazolidinedione-associated congestive heart failure and pulmonary edema. *Mayo Clin Proc* **78:**1088-1091.

Kirchheiner J, Thomas S, Bauer S, Tomalik-Scharte D, Hering U, Doroshyenko O, Jetter A, Stehle S, Tsahuridu M, Meineke I, Brockmöller J and Fuhr U (2006) Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to CYP2C8 genotype. *Clin Pharmacol Ther* **80:**657-667.

Lin ZJ, Ji W, Desai-Krieger D and Shum L (2003) Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-MS/MS. *J Pharm Biomed Anal* **33:**101-108.

Lääketietokeskus (2007) *Pharmaca Fennica 2007 II*. Painoyhtymä Oy, Porvoo, Finland.

Niemi M, Backman JT, Granfors M, Laitila J, Neuvonen M and Neuvonen PJ (2003a) Gemfibrozil considerably increases the plasma concentrations of rosiglitazone. *Diabetologia* **46:**1319-1323.

Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivistö KT and Neuvonen PJ (2005) Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther* **77:**468-478.

Niemi M, Backman JT, Neuvonen M and Neuvonen PJ (2003b) Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia* **46:**347-351.

Niemi M, Backman JT and Neuvonen PJ (2004a) Effects of trimethoprim and rifampin on the pharmacokinetics of the cytochrome P450 2C8 substrate rosiglitazone. *Clin Pharmacol Ther* **76:**239-249.

Niemi M, Kajosaari LI, Neuvonen M, Backman JT and Neuvonen PJ (2004b) The CYP2C8 inhibitor trimethoprim increases the plasma concentrations of repaglinide in healthy subjects. *Br J Clin Pharmacol* **57:**441-447.

Niemi M, Leathart JB, Neuvonen M, Backman JT, Daly AK and Neuvonen PJ (2003c) Polymorphism in CYP2C8 is associated with reduced plasma concentrations of repaglinide. *Clin Pharmacol Ther* **74:**380-387.

Nissen SE and Wolski K (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* **356:**2457-2471.

Ogilvie BW, Zhang D, Li W, Rodrigues AD, Gipson AE, Holsapple J, Toren P and Parkinson A (2006) Glucuronidation converts gemfibrozil to a potent,

metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab Dispos* **34:**191-197.

Olkkola KT, Backman JT and Neuvonen PJ (1994) Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* **55:**481-485.

Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ and Kroetz DL (2007) Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. *Clin Pharmacol Ther* **82:**197-203.

Pedersen RS, Damkier P and Brøsen K (2006) The effects of human CYP2C8 genotype and fluvoxamine on the pharmacokinetics of rosiglitazone in healthy subjects. *Br J Clin Pharmacol* **62:**682-689.

Shitara Y, Hirano M, Sato H and Sugiyama Y (2004) Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* **311:**228-236.

Svirbely JE and Pesce AJ (1987) A high performance liquid chromatography method for trimethoprim utilizing solid-phase column extraction. *Ther Drug Monit* **9:**216-220.

Totah RA and Rettie AE (2005) Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* **77:**341-352.

Weber A, Opheim KE, Siber GR, Ericson JF and Smith AL (1983) High-performance liquid chromatographic quantitation of trimethoprim, sulfamethoxazole, and N4-acetylsulfamethoxazole in body fluids. *J Chromatogr* **278:**337-345.

Wen X, Wang JS, Backman JT, Laitila J and Neuvonen PJ (2002) Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. *Drug Metab Dispos* **30:**631-635.

Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, de Faire U and Rane A (2002) Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. *Biochem Biophys Res Commun* **299:**25-28.

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Reprint Requests: Janne T. Backman, MD, Department of Clinical Pharmacology, University of Helsinki, Haartmaninkatu 4, FIN-00290 Helsinki, Finland. E-mail: janne.backman@helsinki.fi

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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 $AUC_{0\text{-}13\ h}$.

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Table 1

Characteristics of subjects.

Subject No.	Sex	Weight (kg)	BMI (kg/m^2)	*CYP2C8* genotype	Trimethoprim C_{max} (μg/ml)	Trimethoprim $AUC_{0\text{-}13\ h}$ (mg·h/l)
1	Female	65	23	**1/*1*	3.6	37.4
2	Male	58	18	**1/*1*	4.2	38.0
3	Male	75	21	**1/*1*	2.5	22.2
4	Female	72	25	**1/*1*	3.3	29.7
5	Male	93	27	**1/*1*	1.2	7.8
6	Female	63	21	**1/*1*	3.2	27.9
7	Male	76	21	**1/*1*	2.2	20.4
8	Female	56	21	**1/*1*	4.1	36.8
Mean±SD		70±12	22±3		3.0±1.0	27.5±10.5
9	Female	60	23	**1/*3*	3.3	26.4
10	Male	83	23	**1/*3*	2.3	22.4
11	Male	70	20	**1/*3*	2.9	26.2
12	Male	62	22	**1/*3*	3.0	29.0
13	Female	62	19	**1/*3*	4.5	41.9
Mean±SD		67±10	22±2		3.2±0.8	29.2±7.5
14	Female	69	23	**3/*3*	3.0	27.8
15	Female	44	18	**3/*3*	4.7	41.7
16	Male	79	25	**3/*3*	2.3	21.3
Mean±SD		64±18	22±4		3.3±1.2	30.3±10.4

BMI, body mass index; C_{max}, peak plasma concentration; $AUC_{0\text{-}13\ h}$, area under the plasma concentration-time curve from time 0 to 13 h after the morning dose of trimethoprim on day 3.

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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).

Variable	Placebo phase (control)	Trimethoprim phase	Trimethoprim phase percent of control, mean (range)	*p* value between phases[a]	*p* value between genotypes[a]
C_{max} (ng/ml)					
**1/*1*	597±115	659±173	110 (65-183)		
**1/*3*	566±123	707±159	125 (93-233)		
**3/*3*	511±213	593±169	116 (89-179)		
mean	571±131	662±162	116 (65-233)	0.104	0.476
$t_{½}$ (h)					
**1/*1*	4.5±0.6	5.6±1.4	126 (98-182)		
**1/*3*	3.4±0.5*	4.7±1.3	140 (111-159)		
**3/*3*	3.3±0.3*	4.5±0.9	139 (110-176)		
mean	3.9±0.8	5.1±1.3	132 (98-182)	<0.001	0.070
$AUC_{0-\infty}$ (mg·h/l)					
**1/*1*	5.02±1.07	6.88±2.27	137 (81-164)		
**1/*3*	3.87±1.15	6.00±1.99	155 (101-228)		
**3/*3*	3.70±1.19	5.18±1.66	140 (97-200)		
mean	4.42±1.21	6.29±2.07	142 (81-228)	<0.001	0.192
$C_{max,adj}$ (ng/ml)					
**1/*1*	592±135	635±96	107 (65-183)		
**1/*3*	542±113	667±97	123 (93-233)		
**3/*3*	445±163	514±25*†	115 (89-179)		
mean	549±137	622±100	113 (65-233)	0.104	0.021
$AUC_{0-\infty,adj}$ (mg·h/l)					
**1/*1*	4.95±0.96	6.60±1.47	133 (81-164)		
**1/*3*	3.67±0.92*	5.70±1.62	155 (101-228)		
**3/*3*	3.25±0.87*	4.45±0.46*	137 (97-200)		
mean	4.23±1.15	5.92±1.56	140 (81-228)	<0.001	0.017

Values are mean±SD, unless otherwise indicated; C_{max}, peak plasma concentration; $t_{½}$, dominant elimination half-life; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; $C_{max,adj}$, peak plasma concentration adjusted for 70 kg body weight; $AUC_{0-\infty,adj}$, 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*.

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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).

Variable	Placebo phase (control)	Trimethoprim phase	Trimethoprim phase percent of control, mean (range)	*p* value between phases[a]	*p* value between genotypes[a]
M-IV					
k_f (h^{-1})					
**1/*1*	0.12±0.043	0.091±0.039	74 (40-124)		
**1/*3*	0.23±0.086**	0.12±0.045	51 (35-71)		
**3/*3*	0.18±0.056	0.11±0.042	62 (37-100)		
mean	0.17±0.075	0.10±0.040	61 (35-124)	<0.001	0.057
C_{max} (ng/ml)					
**1/*1*	271±62.6	253±38.4	94 (62-135)		
**1/*3*	299±54.9	300±47.0	100 (83-160)		
**3/*3*	269±118	313±112	116 (82-189)		
mean	279±68.3	279±60.5	100 (62-189)	0.600	0.448
$t_{½}$ (h)					
**1/*1*	21.2±3.5	22.5±4.0	106 (95-134)		
**1/*3*	18.8±2.8	19.7±3.9	105 (86-129)		
**3/*3*	23.4±6.5	21.8±1.8	93 (78-118)		
mean	20.9±4.0	21.5±3.7	103 (78-134)	0.804	0.343
$AUC_{0-\infty}$ (mg·h/l)					
**1/*1*	11.6±3.08	12.2±3.48	105 (75-126)		
**1/*3*	11.2±1.86	12.6±2.62	113 (99-142)		
**3/*3*	12.3±4.72	14.6±5.26	119 (96-156)		
mean	11.6±2.91	12.8±3.47	110 (75-156)	0.048	0.829
M-IV/pioglitazone $AUC_{0-\infty}$ ratio					
**1/*1*	2.31±0.41	1.79±0.16	77 (64-92)		
**1/*3*	2.99±0.48*	2.16±0.25**	72 (62-98)		
**3/*3*	3.36±0.73**	2.79±0.27***†	83 (77-99)		
mean	2.72±0.64	2.09±0.43	77 (62-99)	<0.001	0.001

Values are mean±SD, unless otherwise indicated; k_f, apparent formation rate constant; C_{max}, peak plasma concentration; $t_{½}$, elimination half-life; $AUC_{0-\infty}$, 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. 1

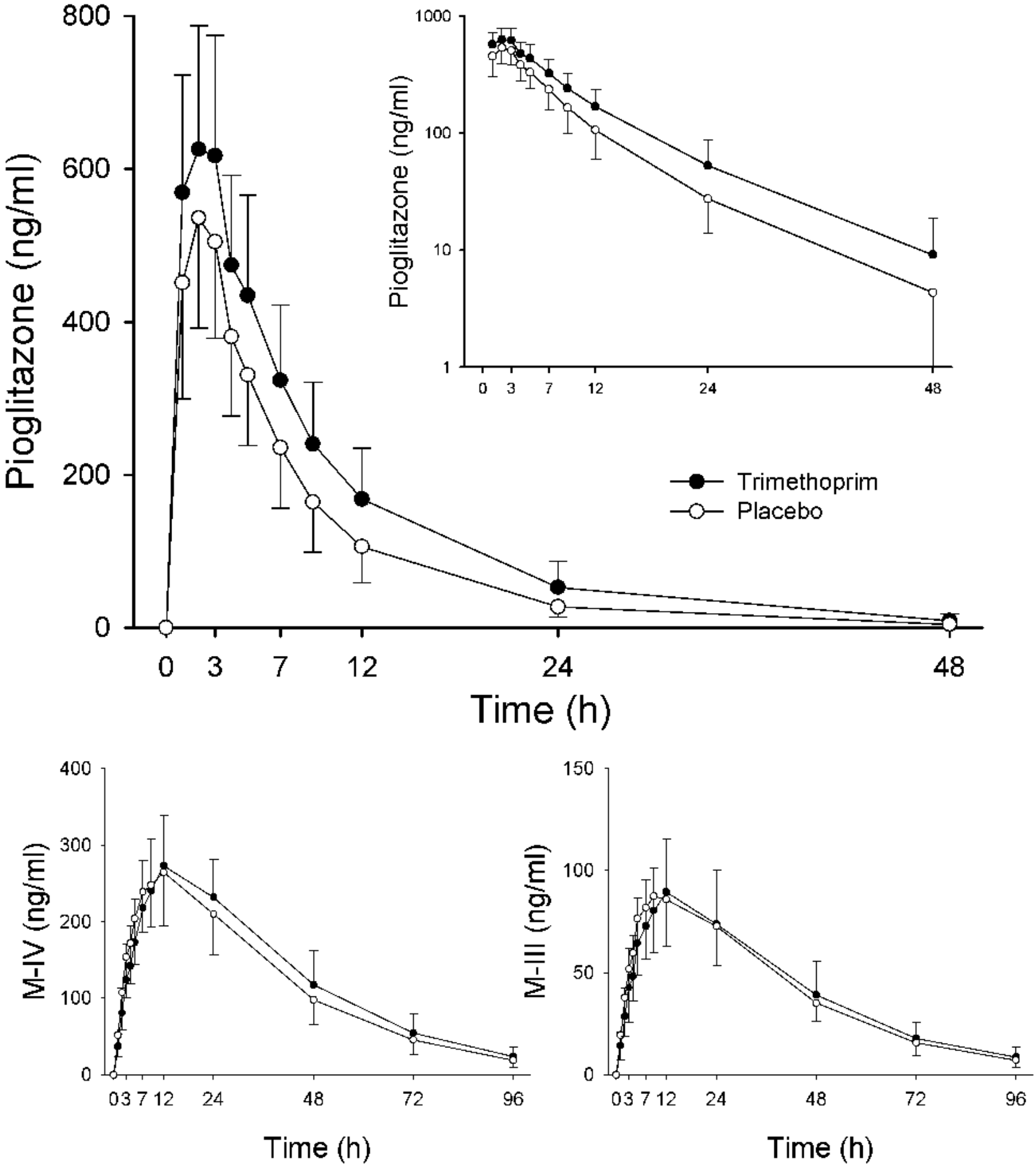

Pioglitazone (ng/ml)
800
600
400
200
0
0 3 7 12 24 48
Time (h)
Trimethoprim
Placebo
Pioglitazone (ng/ml)
1000
100
10
1
0 3 7 12 24 48
M-IV (ng/ml)
400
300
200
100
0
03 7 12 24 48 72 96
Time (h)
M-III (ng/ml)
150
100
50
0
03 7 12 24 48 72 96
Time (h)

Fig. 2

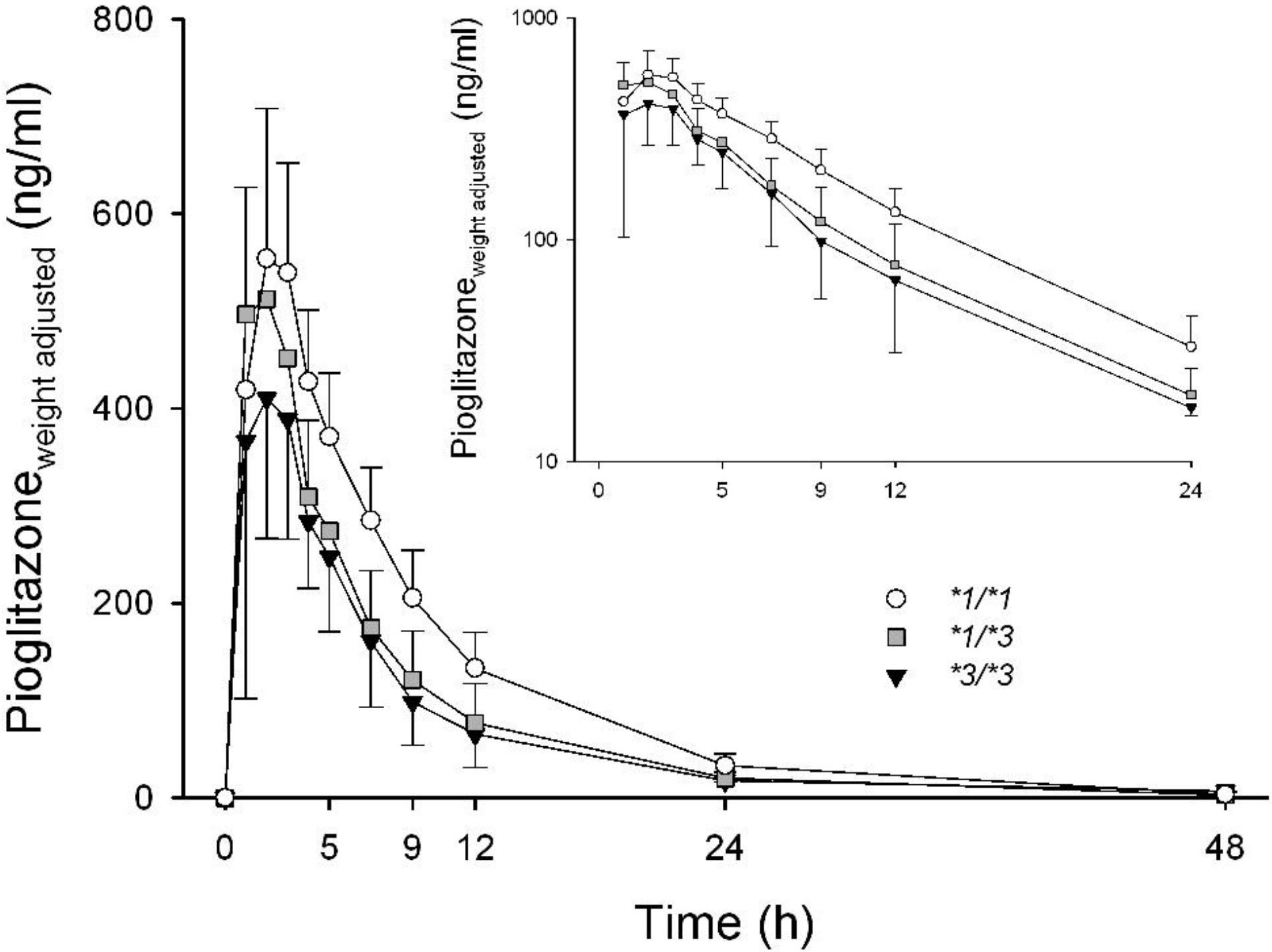
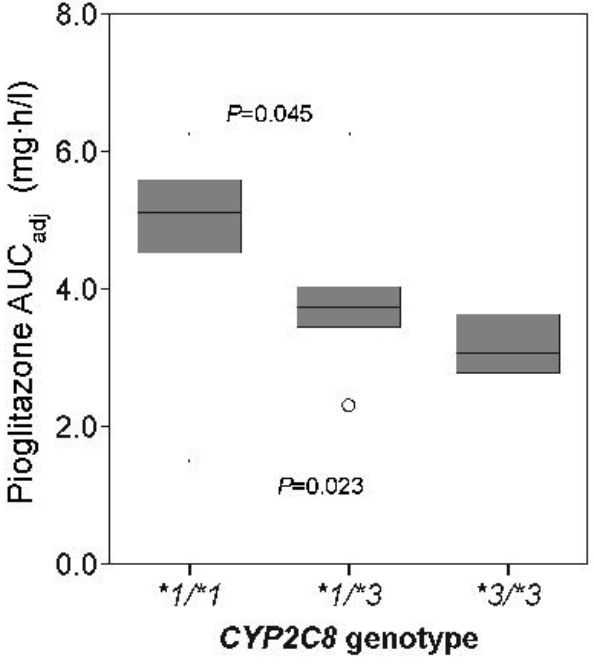
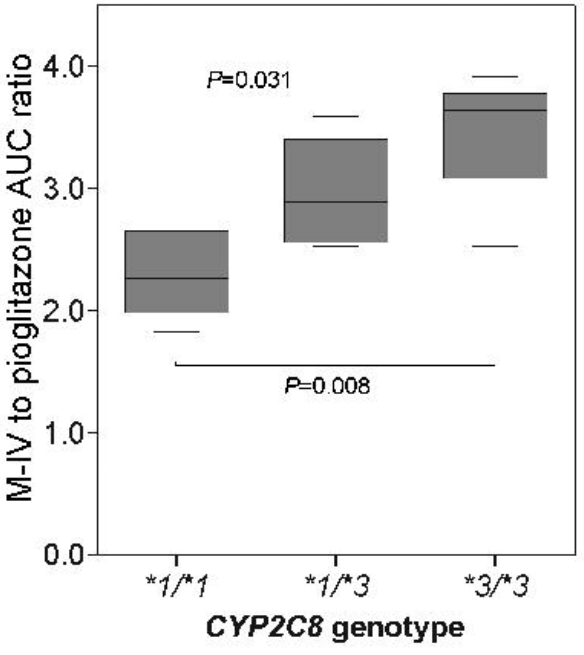

Pioglitazone weight adjusted (ng/ml)
800
600
400
200
0
0
5
9
12
24
48
Time (h)
Pioglitazone weight adjusted (ng/ml)
1000
100
10
0
5
9
12
24
*1/*1
*1/*3
*3/*3

Fig.3


8.0
6.0
4.0
2.0
0.0
Pioglitazone AUC$_{adj}$ (mg·h/l)
P=0.045
P=0.023
*1/*1
*1/*3
*3/*3
CYP2C8 genotype


4.0
3.0
2.0
1.0
0.0
M-IV to pioglitazone AUC ratio
P=0.031
P=0.008
*1/*1
*1/*3
*3/*3
CYP2C8 genotype

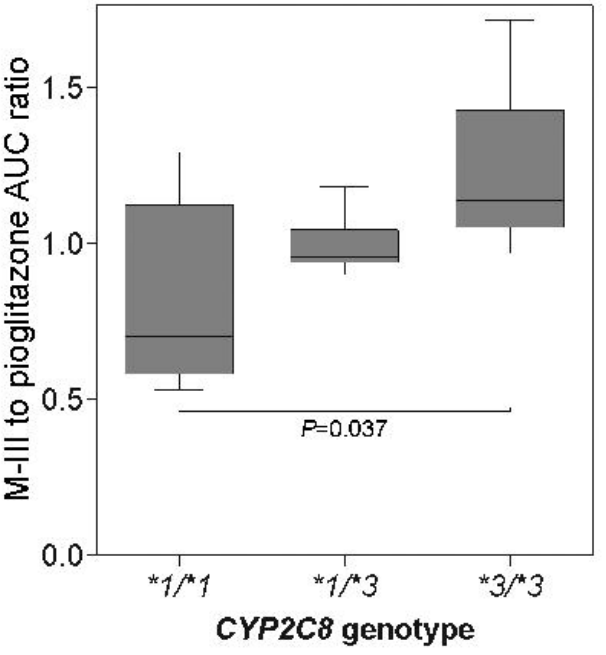

1.5
1.0
0.5
0.0
M-III to pioglitazone AUC ratio
P=0.037
*1/*1
*1/*3
*3/*3
CYP2C8 genotype

Fig. 4

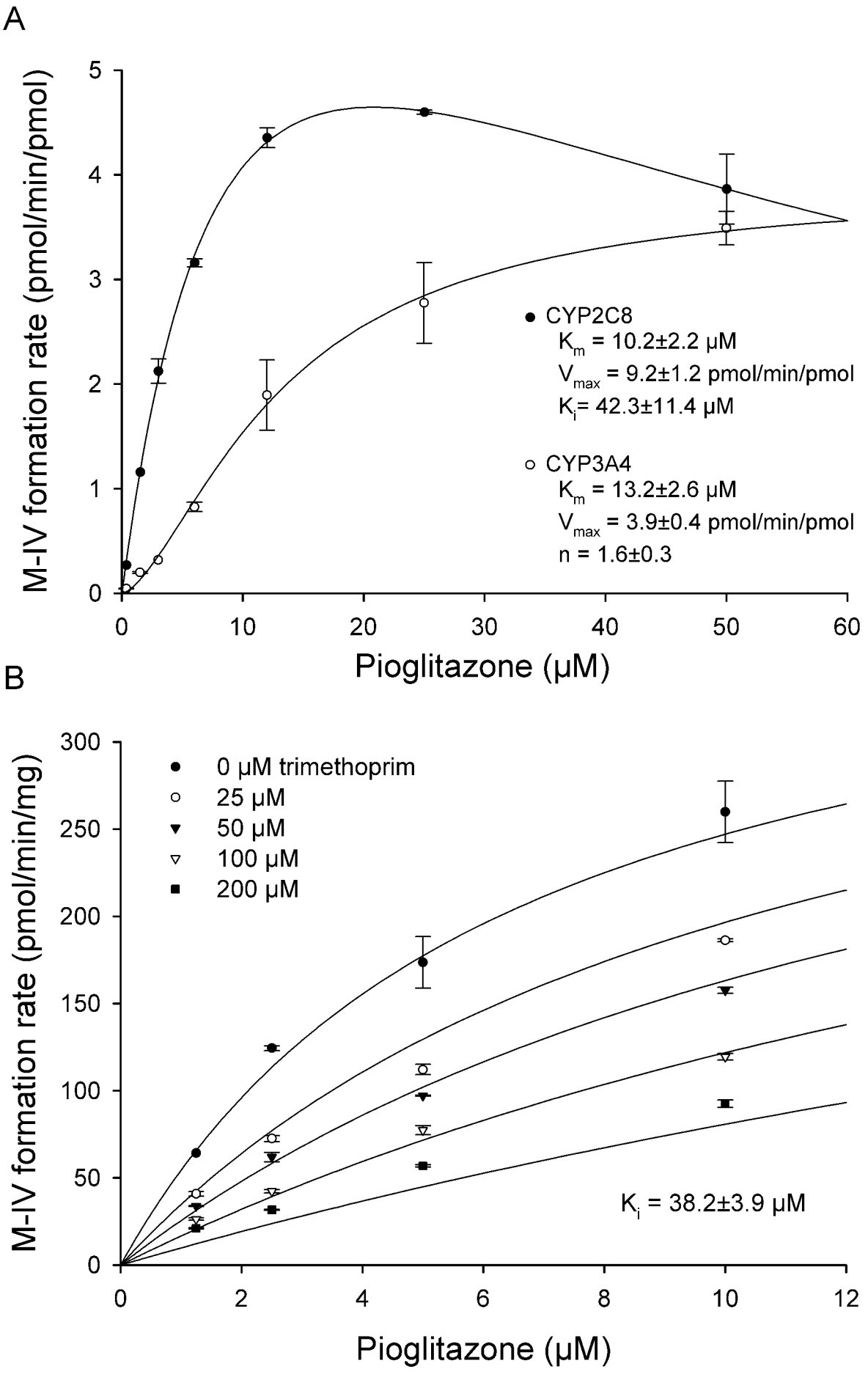


Fig. 5

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

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

$K_i = 38.2\ \mu M$