OXIDATION OF TROGLITAZONE TO A QUINONE-TYPE METABOLITE CATALYZED BY CYTOCHROME P-450 2C8 AND P-450 3A4 IN HUMAN LIVER MICROSOMES

HIROSHI YAMAZAKI, AYAKA SHIBATA, MIKIE SUZUKI, MIKI NAKAJIMA, NORIAKI SHIMADA, F. PETER GUENGERICH, AND TSUYOSHI YOKOI

Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan (H.Y., A.S., M.S., M.N., T.Y.); Daiichi Pure Chemicals, Ibaraki, Japan (N.S.); and Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee (F.P.G.)

(Received for publication March 30, 1999; accepted July 9, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:

Troglitazone, a new oral antidiabetic drug, is reported to be mostly metabolized to its conjugates and not to be oxidized by cytochrome P-450 (P-450) enzymes. Of fourteen cDNA-expressed human P-450 enzymes examined, CYP1A1, CYP2C8, CYP2C19, and CYP3A4 were active in catalyzing formation of a quinone-type metabolite at a concentration of 10 μM troglitazone, whereas CYP3A4 had the highest catalytic activity at 100 μM substrate. In human liver microsomes, rates of the quinone-type metabolite formation (at 100 μM) were correlated well with rates of testosterone 6β-hydroxylation (r = 0.98), but those at 10 μM troglitazone were not correlated with any of several marker activities of P-450 enzymes. Quercetin efficiently inhibited quinone-type metabolite formation (at 10 μM troglitazone) in human samples that contained relatively high levels of CYP2C, whereas ketoconazole affected these activities in liver microsomes in which CYP3A4 levels were relatively high. Anti-CYP2C antibodies strongly inhibited quinone-type metabolite formation (at 10 μM troglitazone) in CYP2C-rich human liver microsomes (by ~85%); the intensity of this effect depended on the human samples and their P-450 status. The results suggest that in human liver both CYP2C8 and CYP3A4 have major roles in quinone-type metabolite formation and that the hepatic contents of these two P-450 forms determine which P-450 enzymes play major roles in individual humans. CYP3A4 may be expected to play a role in formation of quinone-type metabolite from troglitazone even at a low concentration in humans.

Cytochrome P-450 (P-450) comprises a superfamily of enzymes that catalyze oxidation of a great number of xenobiotic chemicals such as drugs, toxic chemicals, and carcinogens as well as endobiotic chemicals, including steroids, fatty acids, prostaglandins, and lipid-soluble vitamins (Guengerich and Shimada, 1991; Guengerich, 1995). In human livers, levels of each of the P-450 forms and roles in various substrate oxidations vary. CYP3A4 is the major P-450 enzyme involved in the oxidation of a large number of compounds (Wrighton and Stevens, 1992; Gonzalez and Gelboin, 1994; Guengerich, 1995).

Troglitazone (Noscal or Rezulin) is a new oral antidiabetic drug recently approved in Japan and the United States for use in the treatment of noninsulin-dependent diabetes mellitus (Sparano and Seaton, 1998). Troglitazone biotransformation has been investigated in rats, mice, dogs, monkeys, and humans, and it is reported to be metabolized mainly to the conjugates shown in Fig. 1, the sulfate (metabolite 1) and glucuronide (metabolite 2) (Izumi et al., 1997a,b; Kawai et al., 1998). In humans, the major products found in plasma are metabolite 1 and, to a lesser extent, a quinone-type metabolite (metabolite 3) (Physicians’ Desk Reference, 1999). Metabolite 3 also was detected in monkeys to a similar extent as in humans but not in rats and mice. Sex differences in pharmacokinetics are observed in rats, i.e., females showed a higher plasma concentration of troglitazone and a lower concentration of metabolite 1 than males, and they excrete a female-specific metabolite, a hydroxylated metabolite 1 (metabolite 4), in the bile (Odaka et al., 1995; Kawai et al., 1997). The oxidized metabolite 3 was found to be further processed to the sulfate in rats (Kawai et al., 1997). Although troglitazone is reported not to be metabolized by human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, and CYP3A4, it has been reported to be a potential inducer of CYP3A4 at clinically relevant concentrations (Physicians’ Desk Reference, 1999) and to significantly decrease cyclosporine (Kaplan et al., 1998), terfenadine, and ethynylestradiol (Koup et al., 1998; Physicians’ Desk Reference, 1999) concentrations in humans. With regard to roles of P-450 enzymes involved in troglitazone metabolism, there is (only) one report that rat CYP2C12 has been shown to catalyze the hydroxylation of metabolite 1 to metabolite 4 (Odaka et al., 1995). Roles of P-450 enzymes in the oxidation of troglitazone to the quinone-type metabolite 3 in human liver are not clear.

Rare cases of severe idiosyncratic hepatocellular injury during marketed use of troglitazone have been reported (Watkins and Whitcomb, 1998; Physicians’ Desk Reference, 1999). The hepatic injury is usually reversible, but very rare cases of hepatic failure, leading to
death or liver transplant, have been reported. Injury has occurred after both short- and long-term troglitazone treatment in the United States (Physicians' Desk Reference, 1999) and after troglitazone treatment for a period ≥4 weeks in Japan (Kuramoto et al., 1998). Hepatic toxicity of troglitazone was not observed in any experimental animals tested, including monkeys, which showed similar metabolite profiles as humans (Summary Basis for Approvals, 1997). In general, quinone-type metabolites are considered to be active intermediates in drug-induced hepatic toxicity after metabolic activation in examples such as acetaminophen, halothane, and diclofenac (Pumford and Halmes, 1997; Bort et al., 1999). It is important to elucidate the mechanism of hepatic toxicity of troglitazone for its safe use. The present study was, therefore, undertaken to determine which P-450 enzymes are most effective in the formation of the quinone-type metabolite (metabolite 3) of troglitazone. Initial studies were performed with recombinant human P-450 enzymes in different expression systems and further studies were done with human liver microsomes to put the work into perspective.

Materials and Methods

Enzyme Preparations. Human liver microsomes were prepared in 10 mM Tris-HCl buffer (pH 7.4) containing 0.10 mM EDTA and 20% glycerol (v/v) as described previously (Shimada and Yamazaki, 1998). Liver samples HL-3, -4, and -10 corresponded to those designated HL-110, -111, and -136 (Guengerich, 1995) and HL-C6, -C15, and -C19 (Yamazaki et al., 1998), respectively. These three microsomal preparations contained total spectrally determined P-450 levels (nanomoles per milligram microsomal protein) of 0.53, 0.32, and 0.45, respectively. Microsomal sample HL-3 had CYP2C9, CYP2C19, and CYP3A4 levels of 12, 0.7, and 73% total P-450, respectively, as judged by immunoblot analysis. Sample HL-4 contained CYP2C9, CYP2C19, and CYP3A4 levels of 0.53, 0.32, and 0.45, respectively. After centrifugation at 900 g for 10 min, product formation in the supernatant was determined by HPLC with a C18 analytical column (4.6 × 150 mm, YMC-Pack A-302; YMC Co. Ltd., Kyoto, Japan). The elution was determined by HPLC with a C18 (5 μm) analytical column (4.6 × 150 mm, YMC-Pack A-302; YMC Co. Ltd., Kyoto, Japan). The elution was determined by HPLC with a C18 (5 μm) analytical column (4.6 × 150 mm, YMC-Pack A-302; YMC Co. Ltd., Kyoto, Japan). The elution was determined by HPLC with a C18 (5 μm) analytical column (4.6 × 150 mm, YMC-Pack A-302; YMC Co. Ltd., Kyoto, Japan). The elution was determined by HPLC with a C18 (5 μm) analytical column (4.6 × 150 mm, YMC-Pack A-302; YMC Co. Ltd., Kyoto, Japan).}

Other Assays. Concentrations of P-450 and b5 (Omura and Sato, 1964) and protein (Lowry et al., 1951) were estimated as described. The contents of P-450 enzymes in human liver microsomes were estimated by coupled SDS-
polyacrylamide gel electrophoresis/immunochemical development (Western blotting) (Guengerich et al., 1982).

Kinetic analysis for substrate oxidations by P-450 enzymes were estimated with a computer program (KaleidaGraph; Synergy Software, Reading, PA) designed for nonlinear regression analysis.

Results

Metabolite 3 Formation Catalyzed by Recombinant P-450 Enzymes Expressed in Different Expression Systems. Formation of metabolite 3 from troglitazone was investigated with recombinant P-450 enzymes coexpressed with NADPH-P-450 reductase in the presence of an NADPH-generating system or CuOOH. Typical chromatograms are shown in Fig. 2. After incubation of troglitazone with CYP2C8, CYP3A4 (Supersomes; Gentest), and human liver microsomes (a sample of HL-4), formation of metabolite 3 was observed. CYP3A4-mediated formation of metabolite 3 from troglitazone also was confirmed with CuOOH as an oxygen surrogate (Fig. 2C); non-enzymatic conversion of troglitazone to metabolite 3 by CuOOH was not observed.

Because troglitazone has been reported not to be metabolized by recombinant CYP3A4 (Physicians' Desk Reference, 1999), we investigated CYP3A4-catalyzed metabolite 3 formation in different expression systems (Fig. 3). Microsomes of lymphoblastoid cells containing CYP3A4 coexpressed with P-450 reductase showed little metabolite 3 formation, although CYP3A4 expressed in two kinds of insect cell microsomes with baculovirus systems or in E. coli membranes catalyzed metabolite 3 formation in the presence of an NADPH-generating system. In a reconstituted system containing purified CYP3A4, metabolite 3 formation was observed in the presence of NADPH or CuOOH (Table 1). Rates for metabolite 3 formation in the reconstituted systems were lower than those in insect cell microsomes expressing CYP3A4, P-450 reductase, and b5 (Supersomes; Gentest).

Metabolite 3 Formation Catalyzed by Recombinant P-450 Enzymes Expressed in Baculovirus Systems. Fourteen forms of recombinant human P-450 enzymes expressed in baculovirus systems with human NADPH-P-450 reductase were used to compare which P-450 forms are active in catalyzing metabolite 3 formation at substrate concentrations of 10 and 100 μM (Fig. 4) (selected on the basis of \( K_m = 28 \mu M \) in liver microsomes vide infra). At 10 μM troglitazone, CYP1A1, CYP3A4, CYP2C8, CYP3A5, and CYP2C19 were highly active in converting troglitazone to metabolite 3 (Fig. 4A). All cDNA-expressed human P-450 enzymes examined had some measurable activity. When the substrate concentration was increased to 100 μM, CYP3A4 had the highest catalytic activity (Fig. 4B).

The above-mentioned results suggest that the major P-450 enzymes...
involved in the troglitazone oxidation in human liver may be CYP2C and CYP3A. Kinetic analysis of the troglitazone oxidation by recombinant P-450 enzymes showed that CYP2C8, CYP2C9, CYP2C19, and CYP3A4 had respective $K_m$ values of 2.7, 3.6, 3.1, and 120 $\mu M$ and $V_{\text{max}}$ values of 4.2, 0.6, 2.8, and 47 nmol/min/nmol P-450 (Table 2).

**Characterization of Quinone-Type Metabolite 3 Formation in Human Liver Microsomes.** Formation of metabolite 3 from troglitazone in standard reaction mixtures containing human liver microsomes was increased linearly with microsomal protein concentration up to 2.0 mg/ml and with time up to 30 min (Fig. 5). Metabolite 3 formation was increased in a substrate concentration-dependent manner, with a hyperbolic plot and some inhibition at high concentrations.

Correlations between marker drug oxidation activities and rates of metabolite 3 formation from troglitazone were analyzed with 10 human liver microsomal samples (Fig. 6). Two substrate concentrations, 10 (Fig. 6, A–D) and 100 $\mu M$ (Fig. 6E), were used. Activities of paclitaxel 6\'-hydroxylation, tolbutamide methyl hydroxylation, S-mephenytoin 4\'-hydroxylation, and testosterone 6\'-hydroxylation did not show good correlations with activities of troglitazone metabolite 3 formation at 10 $\mu M$ concentrations in these liver microsomes.

**TABLE 1**

Rates of quinone-type metabolite 3 formation from troglitazone catalyzed by recombinant CYP3A4 in the presence of an NADPH-generating system or CuOOH

Troglitazone (30 $\mu M$) was incubated at 37°C in the presence of 0.025 $\mu M$ recombinant CYP3A4 and an NADPH-generating system (for 20 min) or 0.1 mM CuOOH (for 5 min) as described in Materials and Methods. Results are represented as means of duplicate determinations.

<table>
<thead>
<tr>
<th>CYP3A4</th>
<th>Metabolite 3 Formation</th>
<th>NADPH-generating system</th>
<th>CuOOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supersomes</td>
<td>11</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Purified CYP3A4</td>
<td>2.8</td>
<td>6.6</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 4.** Metabolite 3 formation from troglitazone at 10 (A) or 100 $\mu M$ (B) catalyzed by recombinant P-450 enzymes.

Troglitazone was incubated for 20 min with recombinant P-450 systems (0.025 $\mu M$ P-450, Supersomes; Gentest) coexpressing NADPH-P-450 reductase in the presence of an NADPH-generating system.

However, at high substrate concentration (100 $\mu M$), metabolite 3 formation activities were highly correlated ($r = 0.98$) with the testosterone 6\'-hydroxylation activities.

Kinetic analysis of metabolite 3 formation in human liver microsomal samples HL-4 and HL-10, which had relatively high contents of CYP2C enzymes (Yamazaki et al., 1998), revealed apparent single $K_m$ and $V_{\text{max}}$ values of 29 $\mu M$ and 143 pmol/min/mg protein and 28 $\mu M$ and 281 pmol/min/mg protein, respectively (Fig. 7). HL-3, with a relatively high content of CYP3A4 (Yamazaki et al., 1998), gave a curve with positive cooperativity and apparently high $K_m$ (435 $\mu M$) and $V_{\text{max}}$ (3040 pmol/min/mg protein) values.

**Effects of P-450 Inhibitors and Anti-CYP Antibodies on Metabolite 3 Formation in Different Human Liver Microsomes.** Effects of P-450 inhibitors on metabolite 3 formation activities catalyzed by liver microsomes of HL-3 and HL-4 were determined at substrate concentrations of 10 and 30 $\mu M$ troglitazone, respectively (Table 3). At a low substrate concentration, quercetin was effective in inhibiting metabolite 3 formation by human liver microsomes (sample HL-4). However, when 30 $\mu M$ troglitazone was used, quercetin did not inhibit, even in sample HL-4. Ketoconazole inhibited metabolite 3 formation in both human liver microsomal samples at both substrate concentrations, but neither sulfaphenazole, fluvoxamine, nor quinidine inhibited metabolite 3 formation (Table 3). Inhibition of metabolite 3 formation was more extensive with a combination of quercetin and ketoconazole in human liver microsomal samples. In separate experiments, quercetin (10 $\mu M$) inhibited paclitaxel 6\'-hydroxylation (at 10 $\mu M$ substrate concentration) by ~40% in human liver microsomal sample HL-4 (data not shown).

Anti-CYP2C IgG strongly inhibited microsomal troglitazone oxidation activities at 10 $\mu M$ in human liver microsomal sample HL-4, although the inhibitory effect of anti-CYP2C antibodies was less than that of anti-CYP3A4 antibodies in microsomal sample HL-3 (Table 4).

**Discussion**

Troglitazone is a new oral hypoglycemic agent recently approved for use in type II diabetes mellitus. Troglitazone has a vitamin E-like structure (hydroxychroman moiety) and has been suggested to be nonenzymatically converted to a quinone-type metabolite by lipid peroxides or active oxygen (Fu et al., 1996). Although troglitazone is a potential inducer of CYP3A4 (Kaplan et al., 1998; Koup et al., 1998), it is reported not to be metabolized by CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, and CYP3A4 (Physicians' Desk Reference, 1999). Why has this information (Physicians' Desk Reference, 1999) been reported regarding the roles of P-450 enzymes involved in the oxidation of troglitazone by human liver microsomes? It has been shown that there are variations in catalytic activities of recombinant P-450 enzymes expressed in different systems (Shaw et al., 1997; Yamazaki et al., 1997). In this study, metabolite 3 formation...
FIG. 5. Effects of microsomal protein (A), incubation time (B), and substrate concentration (C) on quinone-type metabolite 3 formation catalyzed by human liver microsomes (sample HL-4).

The basic incubation mixture was used. In (A) and (B), the concentration of troglitazone was 100 μM; in (B) and (C), the microsomal protein concentration was 1.0 mg/ml; in (A) and (C), the mixtures were incubated for 20 min in the presence of an NADPH-generating system.

FIG. 6. Relationship between rates for metabolite 3 formation from troglitazone at 10 (A–D) or 100 μM (E) and drug oxidation activities in different human liver microsomes.

FIG. 6. Relationship between rates for metabolite 3 formation from troglitazone at 10 (A–D) or 100 μM (E) and drug oxidation activities in different human liver microsomes.
from troglitazone catalyzed by CYP3A4 was clearly shown (in a substrate-dependent manner) in the presence of NADPH or CuOOH, whereas microsomes of lymphoblastoid cells did not produce detectable amounts of metabolite 3. These findings may be related to the false negative reports (Physicians’ Desk Reference, 1999). It should be mentioned that the choice of recombinant P-450 enzyme systems is of great importance for drug metabolism research, and sensitivity may be an issue in this case. The reason why we focused on quinone-type metabolite 3 has been detected in human plasma after oral administration of troglitazone, and quinone-type metabolites are generally considered to be active intermediates in drug metabolism. The reason why we focused the quinone-type formation at 10 μM troglitazone was not recovered in the absence or presence of an NADPH-generating system, similar to findings reported previously (Izumi et al., 1997b). We then chose the low- and high-substrate concentrations at 10 and 100 μM troglitazone, respectively, for further work.

Correlation analysis suggested that at least two or more P-450 enzymes in human liver microsomes might catalyze metabolite 3 formation at 10 μM troglitazone because no clear patterns were found. CYP3A4 would be a major catalyst at higher substrate concentrations. At 30 μM (an apparent single K_m value in human liver microsomes), CYP3A4 was also a major enzyme involved in metabolite 3 formation because chemical inhibition was observed only with ketoconazole at this substrate concentration. Average levels of CYP3A4 in human livers have been determined to be ~30% of total P-450 in Japanese and Caucasian samples examined; in some people CYP3A4 level accounts for >60% of total P-450, probably due to the induction by various chemical agents (Guengerich, 1995). The average content of CYP2C19 in human liver microsomes has been reported to be ~1% of total P-450, and contents of CYP2C8 were much lower than those of CYP2C19 (Inoue et al., 1997). These findings support the idea that the contribution of P-450 enzymes in troglitazone oxidation reactions are 0.9 to 2.8 μg/ml (2.0–6.4 μM) in the steady state in normal volunteers (Physicians’ Desk Reference, 1999). In our preliminary experiments with microsomal protein of human liver and lymphoblastoid cells expressing CYP3A4, approximately half of an initial concentration of 5 μM troglitazone was not recovered in the absence or presence of an NADPH-generating system, similar to findings reported previously (Izumi et al., 1997b). Then we chose the low- and high-substrate concentrations at 10 and 100 μM troglitazone, respectively, for further work.

Correlation analysis suggested that at least two or more P-450 enzymes in human liver microsomes might catalyze metabolite 3 formation at 10 μM troglitazone because no clear patterns were found. CYP3A4 would be a major catalyst at higher substrate concentrations. At 30 μM (an apparent single K_m value in human liver microsomes), CYP3A4 was also a major enzyme involved in metabolite 3 formation because chemical inhibition was observed only with ketoconazole at this substrate concentration. Average levels of CYP3A4 in human livers have been determined to be ~30% of total P-450 in Japanese and Caucasian samples examined; in some people CYP3A4 level accounts for >60% of total P-450, probably due to the induction by various chemical agents (Guengerich, 1995). The average content of CYP2C19 in human liver microsomes has been reported to be ~1% of total P-450, and contents of CYP2C8 were much lower than those of CYP2C19 (Inoue et al., 1997). These findings support the idea that the contribution of P-450 enzymes in troglitazone oxidation reactions are 0.9 to 2.8 μg/ml (2.0–6.4 μM) in the steady state in normal volunteers (Physicians’ Desk Reference, 1999). In our preliminary experiments with microsomal protein of human liver and lymphoblastoid cells expressing CYP3A4, approximately half of an initial concentration of 5 μM troglitazone was not recovered in the absence or presence of an NADPH-generating system, similar to findings reported previously (Izumi et al., 1997b). We then chose the low- and high-substrate concentrations at 10 and 100 μM troglitazone, respectively, for further work.

TABLE 4

<table>
<thead>
<tr>
<th>Antibody</th>
<th>mg IgG/μmol P-450</th>
<th>Metabolite 3 Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/min/μmol P-450</td>
<td>HL-3</td>
</tr>
<tr>
<td>None</td>
<td>114 (100)</td>
<td>123 (100)</td>
</tr>
<tr>
<td>Preimmune</td>
<td>90 (79)</td>
<td>96 (75)</td>
</tr>
<tr>
<td>Anti-CYP2C</td>
<td>73 (64)</td>
<td>18 (14)</td>
</tr>
<tr>
<td>Anti-CYP3A</td>
<td>63 (55)</td>
<td>17 (13)</td>
</tr>
<tr>
<td>Anti-CYP3A</td>
<td>52 (46)</td>
<td>76 (62)</td>
</tr>
<tr>
<td>Anti-CYP3A</td>
<td>41 (36)</td>
<td>68 (55)</td>
</tr>
</tbody>
</table>

TABLE 3

<table>
<thead>
<tr>
<th>Addition</th>
<th>μM</th>
<th>10 μM troglitazone</th>
<th>30 μM troglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HL-3</td>
<td>HL-4</td>
</tr>
<tr>
<td>None</td>
<td>191 + 18 (100)</td>
<td>138 + 18 (100)</td>
<td>292 (100)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>204 + 14 (107)</td>
<td>80 + 10 (58)</td>
<td>387 (133)</td>
</tr>
<tr>
<td>Sulfaphenazole</td>
<td>228 + 16 (119)</td>
<td>124 + 9 (90)</td>
<td>428 (147)</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>236 + 21 (124)</td>
<td>137 + 11 (99)</td>
<td>443 (152)</td>
</tr>
<tr>
<td>Quinidine</td>
<td>211 + 10 (110)</td>
<td>141 + 13 (102)</td>
<td>319 (109)</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>130 + 11 (68)</td>
<td>76 + 6 (55)</td>
<td>157 (54)</td>
</tr>
<tr>
<td>Combination of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quercetin and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ketoconazole</td>
<td>92 + 14 (48)</td>
<td>44 + 16 (32)</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate percentage of control (none): n.d., not done.

*p < .05, **p < .01.
in human livers may be altered by using different human samples with compositions of various P-450 enzymes in the liver. Using different human samples that contain varying levels of individual P-450 enzymes in the liver and recombinant human P-450 enzymes expressed in various systems, we obtained several lines of evidence to support the view that different human P-450 enzymes, particularly CYP2C8 and CYP3A4, contribute significantly to troglitazone oxidation to the quinone-type metabolite in humans and that the roles of these P-450 enzymes vary with the use of different human samples. The results obtained in this study can be summarized as follows. In human livers having relatively high contents of CYP2C and low CYP3A4 (a sample HL-4), anti-CYP2C and quercetin, an inhibitor against CYP2C8 (Rahman et al., 1994), suppressed this reaction significantly at low substrate concentrations (and recombinant CYP2C8 had a higher vmax/Km ratio than CYP3A4). However, the role of CYP3A4 is much greater in human samples that contain relatively high levels of CYP3A4, e.g., sample HL-3, and this reaction was inhibited by ketoconazole and anti-CYP3A4 even at low substrate concentrations. Apparently the Km component (~30 μM) observed in human liver microsomes was higher that those of recombinant CYP2C enzymes (~3 μM). No inhibitory effects of fluvoxamine, an inhibitor of both CYP1A2 and CYP2C19 (Jeppesen et al., 1996; Yamazaki et al., 1997); sulfaphenazole, an inhibitor of CYP2C9 (Mancy et al., 1996); or quinidine, an inhibitor of CYP2D6 (Otton et al., 1984), on metabolite 3 formation were observed, suggesting that CYP2C9 and CYP2C19 as well as CYP1A2 and CYP2D6 play only minor roles in metabolite 3 formation in human liver microsomes. In conclusion, our results suggest that both CYP2C8 and CYP3A4 are major P-450 enzymes involved in the oxidation of troglitazone to a quinone-type metabolite in human livers. The roles of these two P-450s vary depending on the contents of CYP2C8 and CYP3A4 in human liver microsomes. In general, CYP3A4 has the highest content of any P-450s in the liver and other CYP3A4-rich sites, CYP2C8 may also play an essential contribution. This information about the roles of individual human P-450 enzymes in troglitazone oxidations is of relevance in evaluating hepatocellular injury in troglitazone treatment in humans. Studies of drug interaction caused by troglitazone via inhibition of P-450-supported drug metabolism is under investigation.

Acknowledgments. We thank Sankyo for providing troglitazone and its quinone-type metabolite 3 used in this study.

References