The troglitazone glucuronidation in human liver and jejunum microsomes also exhibited an $V_{\text{max}}$ value of 34.8 pmol/min/mg protein. The kinetics of the troglitazone glucuronidation in the recombinant UGT1A10 and UGT1A1 exhibited an atypical pattern of substrate inhibition when the substrate concentration was over 200 $\mu$M. With a Michaelis-Menten equation at 6 to 200 $\mu$M troglitazone, the $K_m$ value was 11.1 ± 5.8 $\mu$M and the $V_{\text{max}}$ value was 33.6 ± 3.7 pmol/min/mg protein in recombinant UGT1A10. In recombinant UGT1A1, the $K_m$ value was 58.3 ± 29.2 $\mu$M and the $V_{\text{max}}$ value was 12.3 ± 2.5 pmol/min/mg protein. The kinetics of the troglitazone glucuronidation in human intestine and jejunum microsomes also exhibited an atypical pattern. The $K_m$ value was 13.5 ± 2.0 $\mu$M and the $V_{\text{max}}$ value was 34.8 ± 1.2 pmol/min/mg for troglitazone glucuronidation in human liver microsomes, and the $K_m$ value was 6.1 ± 0.3 $\mu$M and the $V_{\text{max}}$ value was 700.9 ± 4.3 pmol/min/mg protein in human jejunum microsomes. When the intrinsic clearance was estimated with the in vitro kinetic parameter, mesosomal protein content, and weight of tissue, troglitazone glucuronidation in human intestine was 1.9-fold higher than that in human livers. Interindividual differences in the troglitazone glucuronosyltransferase activity in liver microsomes from 13 humans were at most 2.2-fold. The troglitazone glucuronosyltransferase activity was significantly ($r = 0.579, p < 0.05$) correlated with the $\beta$-estradiol 3-glucuronosyltransferase activity, which is mainly catalyzed by UGT1A1. The troglitazone glucuronosyltransferase activity in pooled human liver microsomes was strongly inhibited by bilirubin (IC$_{50}$ = 1.9 $\mu$M), a typical substrate of UGT1A1. These results suggested that the troglitazone glucuronidation in human liver would be mainly catalyzed by UGT1A1. Interindividual differences in the troglitazone glucuronosyltransferase activity in S-9 samples from five human intestines was 8.2-fold. The troglitazone glucuronosyltransferase activity in human jejunum microsomes was strongly inhibited by emodin (IC$_{50}$ = 15.8 $\mu$M), a typical substrate of UGT1A8 and UGT1A10, rather than by bilirubin (IC$_{50}$ = 154.0 $\mu$M). Therefore, it is suggested that the troglitazone glucuronidation in human intestine might be mainly catalyzed by UGT1A8 and UGT1A10.

Troglitazone is an antidiabetic agent that increases the insulin sensitivity of target tissues in noninsulin-dependent diabetes mellitus (Nolan et al., 1994; Fujiwara et al., 1998). Although troglitazone-associated hepatitis is thought to be rare, there are clinical reports of severe hepatic reactions associated with the use of troglitazone. About 1.9% of patients experience an increase in alanine aminotransferase associated hepatitis is thought to be rare, there are clinical reports of severe hepatic reactions associated with the use of troglitazone. About 1.9% of patients experience an increase in alanine aminotransferase for a period longer than 4 weeks in Japan (Kuramoto et al., 1998). The mechanism by which troglitazone causes liver dysfunction in certain individuals is still unclear. Recently, it has been reported that the inhibition of troglitazone sulfation, which is recognized as a major metabolic pathway (Loi et al., 1999), may result in...
increased hepatotoxicity due to exposure to the parent drug or increased metabolism by alternate pathways (Kostrubsky et al., 2000).

Glucuronidation is catalyzed by UGT (Miners and Mackenzie, 1991). It is well known that there are many isoforms of mammalian UGT enzymes (Tukey and Strassburg, 2000). Human UGTs are expressed in a tissue-specific manner in hepatic and extrahepatic tissues (Strassburg et al., 1997). The distribution of individual UGT isoforms is believed to determine tissue-specific metabolism and detoxification. UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B8, UGT2B10, UGT2B15, and UGT2B17 are expressed in the liver (Burchell et al., 2001). UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A10, UGT2B7, UGT2B10, and UGT2B15 are expressed in human intestine (Strassburg et al., 2000; Tukey and Strassburg, 2000; Burchell et al., 2001). Until now, in humans, the troglitazone glucuronosyltransferase activity has been studied only in the liver (Yoshigae et al., 2000). It has been reported that troglitazone inhibits bilirubin glucuronidation (catalyzed by UGT1A1) and 1-naphthol glucuronidation (mainly catalyzed by UGT1A6) in human liver microsomes (Yoshigae et al., 2000; Ito et al., 2001). Furthermore, it has also been reported that recombinant UGT1A1, UGT1A4, UGT1A6, and UGT1A9 in microsomes from human B-lymphoblastoid cells have a capacity for troglitazone glucuronidation (Yoshigae et al., 2000). In the present study, the troglitazone glucuronosyltransferase activities in human intestinal S-9 and jejunal microsomes were compared with those in human liver microsomes. We first demonstrated that human jejunal microsomes exhibit prominently higher troglitazone glucuronosyltransferase activity than human liver microsomes. To identify the human UGT isoform(s) responsible for troglitazone glucuronidation, the activities in commercially available recombinant UGTs in microsomes from baculo-virus-infected insect cells were determined. Furthermore, an inhibition analysis and correlation analysis using human liver and jejunal microsomes were performed.

Materials and Methods

Materials. Troglitazone and troglitazone glucuronide were kindly provided by Sankyo (Tokyo, Japan). UDP-glucuronic acid, alamethicin, β-estradiol, emodin, p-nitrophenyl β-glucuronide, and α-naphthyl β-glucuronide were purchased from Sigma-Aldrich (St. Louis, MO). Bilirubin, 4-nitrophenol, 1-naphthol, and imipramine hydrochloride were from Wako Pure Chemicals (Osaka, Japan). Morphine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). Morphine-3-glucuronide was kindly provided by Dr. Katzura Oguri (Kyusyu University, Fukuoka, Japan). Pooled human liver microsomes (H161) and microsomes from 13 individual human liver samples (H161-48, H161-50, H161-51, H161-72, H161-73, H161-76, H161-77, H161-78, H161-100, H161-101, H161-105, H161-109, and H161-110) were purchased from BD Gentest (Woburn, MA). The glucuronosyltransferase activities of β-estradiol and bilirubin in these human liver microsomes were provided as typical activities for UGT1A1 by the manufacturer. The glucuronosyltransferase activities of trifluoperazine and propofol in these human liver microsomes were also provided as typical activities for UGT1A4 and UGT1A9, respectively. Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15 expressed in baculovirus-infected insect cells (Supersomes) and control microsomes from insect cells infected with wild-type baculovirus were also from BD Gentest. Recombinant UGT1A1, UGT1A3, UGT1A6, UGT1A7, and UGT1A10 expressed in baculovirus-infected insect cells (Baculosomes) were from PanVera (Madison, WI). Polyclonal rabbit anti-human UGT1A antibodies were from BD Gentest. The human intestine specimens were provided by National Disease Research Interchange (Philadelphia, PA) through HAB Discussion Group (Chiba, Japan) according to the International Partnership between both nonprofit organizations. The intestinal S-9 fractions (HIS-052, HIS-053, HIS-078, HIS-084, and HIS-111) were prepared by HAB Biomedical Research Institute (Chiba, Japan). The human jejunal microsomes (HJM0040) were obtained from KAC (Shiga, Japan). All other chemicals and solvents were of the highest grade commercially available.

Troglitazone Glucuronidation Assay. A typical incubation mixture (200-μl total volume) contained 25 mM Tris-HCl buffer, pH 7.4, 5 mM MgCl₂, 3 mM UDP-glucuronic acid, 50 μg of alamethicin/mg microsomal, 1.0 mg/ml human liver microsomes (2 mg/ml human intestinal S-9, 0.5 mg/ml human jejunal microsomes, or 1 mg/ml for recombinant UGT), and 100 μM troglitazone. The reaction was initiated by the addition of troglitazone and the reaction mixture was incubated for 20 min. The reaction was then terminated by boiling for 10 min. After removal of the protein by centrifugation at 10,000 rpm for 5 min, a 100-μl portion of the sample was subjected to HPLC. Chromatography was performed using an LC-6A pump (Shimadzu, Kyoto, Japan), an SPD-6 UV detector (Shimadzu), an SIL-6B autosampler (Shimadzu), a C-R4A integrator (Shimadzu), and a CTO-6A column oven (Shimadzu) with a YMC-pack A302 column (4.6 × 150 mm, 5 μm; YMC, Tokyo, Japan). The flow rate was 1.5 ml/min and the column temperature was 35°C. The eluate was monitored at 230 nm with a noise-base clean Uni-3 (Union, Gunma, Japan). The Uni-3 can reduce the noise by integrating the output and both increase the signal 3-fold by differentiating the output and an additional 5-fold by further amplification with an internal amplifier, resulting in a maximum 15-fold amplification of the signal. The mobile phase was 39% CH₃CN/0.05% H₃PO₄ (v/v). The retention times of troglitazone glucuronide and troglitazone were 6.2 and 50.0 min, respectively. None of these chromatograms showed any interfering peaks with troglitazone glucuronide (data not shown). The quantification of troglitazone glucuronosyltransferase activities was performed by comparing by the HPLC peak heights to those of authentic standards.

Kinetic Analyses. The kinetics studies were performed using human liver microsomes, recombinant human UGT1A1 and UGT1A10 expressed in baculovirus-infected insect cells (Supersomes), and human jejunal microsomes. In determining the kinetic parameters, the troglitazone concentration ranged from 6 μM to 1 mM. Kinetic parameters were estimated from the fitted curves using a computer program of KaleidaGraph (Synergy Software, Reading, PA) designed for nonlinear regression analysis. The following equations were applied for Michaelis-Menten kinetics, with eq. 1 using the activities at 6 to 200 μM troglitazone or substrate inhibition kinetics, and eq. 2 using the activities at 6 μM to 1 mM troglitazone:

\[ V = V_{max} \cdot \frac{S}{K_m + S} \]  (1)

\[ V = V_{max} \cdot S \left( \frac{K_m + S + S/K_i}{K_m + S + S/K_i} \right) \]  (2)

where \( K_m \) is a Michaelis-Menten constant, \( V_{max} \) is the maximum velocity, and \( K_i \) is the substrate inhibition constant (Houston and Kenworthy, 2000).

Metabolite Inhibition Analysis of Troglitazone Glucuronosyltransferase Activity in Human Liver Microsomes. The inhibitory effects of troglitazone glucuronide per se on the troglitazone glucuronosyltransferase activity in the human liver microsomes were determined. Troglitazone glucuronide was added as an inhibitor to the incubation mixture at a final concentration of 0.06 to 1 μM. Troglitazone glucuronosyltransferase activity in the pooled human liver microsomes at 100 μM troglitazone was calculated with the formed metabolite by subtracting the content of the metabolite added as an inhibitor from the total content of metabolite.

Inhibition Analysis of Troglitazone Glucuronosyltransferase Activity in Human Liver and Jejunum Microsomes. Six compounds were tested for their inhibitory effects on the troglitazone glucuronosyltransferase activity. Bilirubin is a typical substrate for UGT1A1 (Bosma et al., 1994; Senafi et al., 1994; Kao et al., 1996). β-Estradiol is a typical substrate of UGT1A1 (Yoshigae et al., 2000; Senafi et al., 1994; Hanikoa et al., 2001a,b). 4-Nitrophenol is a substrate for UGT1A6 and UGT1A9 (Hanioka et al., 2001a,b). Imipramine is a substrate for UGT1A4 (Yoshigae et al., 2000; Senafi et al., 1994; Hanikoa et al., 2001a,b). Emodin is a substrate for UGT1A8 and UGT1A10 (Cheng et al., 1999). Morphine is a substrate for UGT2B7 (Coffman et al., 1997). For the determination of the IC₅₀ values, the concentration of troglitazone was set at 100 μM. Bilirubin and 4-nitrophenol were dissolved in dimethyl sulfoxide and ethanol, respectively. β-Estradiol and emodin were dissolved in methanol. Imipramine hydrochloride, and morphine hydrochloride were dissolved in water. The final concentration of the organic solvents in the reaction mixture was <1% (v/v). The troglitazone glucuron-
syltransferase activities in pooled human liver microsomes (H161) and in individual human jejunum microsomes (HJM0040) at 100 μM troglitazone were determined as described above.

Other Glucuronidation Assays. 4-Nitrophenol glucuronosyltransferase activity in human liver microsomes was determined as described previously (Hanioka et al., 2001a). Briefly, a typical incubation mixture (200-μl total volume) contained 50 mM potassium phosphate buffer, pH 7.4, 0.2% Triton N-101, 3 mM UDP-glucuronic acid, 1.0 mg/ml human liver microsomes, and 500 μM 4-nitrophenol. The reaction was initiated by the addition of UDP-glucuronic acid and was then incubated at 37°C for 5 min. The reaction was terminated by boiling at 100°C for 2 min and adding 2.8 ml of 0.2 M glycine buffer, pH 10.4. After removal of the protein by centrifugation at 2000 rpm for 20 min, a 50-μl portion of the sample was subjected to HPLC. The HPLC instrument was the same as described above. Chromatographic separations were performed on a Mightysil RP-18 (4.6 × 150 mm; 5 μm) column (Kanto Chemical, Tokyo, Japan). The flow rate was 1.2 ml/min at 35°C. The mobile phase was 5% CH3 OH/0.05M KH 2 PO 4 , pH 6.5. The retention times of morphine 3-glucuronide and morphine were 3.0 and 14.5 min, respectively. Formation of the metabolite was quantified by comparing the peak areas in the incubations to a standard curve containing known amounts of the metabolite.

1-Naphthol glucuronosyltransferase activity in human liver microsomes was determined as described previously (Mackenzie and Hanninen, 1980). Briefly, a typical incubation mixture (200-μl total volume) contained 50 mM potassium phosphate buffer, pH 7.4, 0.2% Triton N-101, 3 mM UDP-glucuronic acid, 0.5 mg/ml human liver microsomes, and 50 μM 1-naphthol. The reaction was initiated by the addition of UDP-glucuronic acid followed by incubation at 37°C for 2.5 min. The reaction was terminated by boiling for 2 min and adding 1.8 ml of phosphate-buffered saline. After removal of the protein by centrifugation at 2000 rpm for 20 min, the fluorescence of the supernatant was measured at excitation 290 nm and emission 343 nm by an RF5000 fluorescence detector (Shimadzu). Formation of the metabolite was quantified by comparing the fluorescence in the incubations to a standard curve containing known amounts of the metabolite.

Morphine glucuronosyltransferase activity in human liver microsomes was determined as described previously (Milne et al., 1991). Briefly, a typical incubation mixture (200-μl total volume) contained 50 mM potassium phosphate buffer, pH 7.4, 0.2% Triton N-101, 3 mM UDP-glucuronic acid, 1.0 mg/ml human liver microsomes, and 1 mM morphine. The reaction was initiated by the addition of UDP-glucuronic acid and was then incubated at 37°C for 30 min. The reaction was terminated by adding 250 μl of ice-cold acetonitrile. After removal of the protein by centrifugation at 2000 rpm for 20 min, a 50-μl portion of the sample was subjected to HPLC. The HPLC instrument was the same as described above. Chromatographic separations were performed on a Mighty sil RP-18 (4.6 × 150 mm; 5 μm) column (Kanto Chemical). The flow rate was 1.2 ml/min and the column temperature was 35°C. The mobile phase was 20% CH3 CN/0.8 mM sodium dodecyl sulfate/0.05 M KH 2 PO 4 , pH 1.9. The eluate was monitored at excitation 210 nm and emission 330 nm. The retention times of morphine 3-glucuronide and morphine were 3.1 and 8.0 min, respectively. Formation of the metabolite was quantified by comparing the peak areas in the incubations to a standard curve containing known amounts of the metabolite.

Immunoblot Analysis of Recombinant UGT 1A Isoforms. SDS-poly acrylamide gel electrophoresis and immunoblot analysis of recombinant UGT1A isoforms were performed according to Laemmli (1970). The microsomes from baculovirus-infected insect cells (2 μg) were separated on 7.5% polyacrylamide gel and transferred electrophoretically to a nitrocellulose membrane. Polyclonal anti-human UGT1A antibodies have been reported to detect human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, and UGT1A10 by the manufacturer. Biotinylated anti-rabbit IgG and VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA) were used for diaminobenzidine staining.

Correlation Analyses. Correlation analyses between troglitazone glucuronidation and the other glucuronidation activities were determined by Pearson’s product moment method. A p value of less than 0.05 was considered statistically significant.

Results

Troglitazone Glucuronidation in Recombinant UGT Isoforms. Ten recombinant UGT isoforms expressed in baculovirus-infected insect cells were used to determine their troglitazone glucuronosyltransferase activities (Fig. 1). In Supersomes, UGT1A10 (31.0 ± 0.2 pmol/min/mg protein) and UGT1A8 (17.6 ± 3.3 pmol/min/mg protein) exhibited high troglitazone glucuronosyltransferase activities (Fig. 1A). All other isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, GT2B7, and UGT2B15) exhibited low troglitazone glucuronosyltransferase activities. In Baculosomes, UGT1A10 (56.4 ± 3.5 pmol/min/mg protein) exhibited the highest troglitazone glucuronosyltransferase activities (Fig. 1B). Other UGTs also exhibited weak glucuronosyltransferase activities.

Immunoblot Analysis of Recombinant UGT 1A Isoforms. In Supersomes, band density with all UGT1A isoforms was almost similar except for UGT1A3. In Baculosomes, UGT1A3, UGT1A6, and UGT1A10 exhibited weak detectable band, although UGT1A1 and UGT1A7 exhibited a clear band.

Kinetics of Troglitazone Glucuronidation in Recombinant UGT1A1 and UGT1A10 (Supersomes). UGT1A1 is a major isofom in human liver (Bock et al., 1999). UGT1A8 and UGT1A10 are both expressed in the intestine but not in the liver, and the substrate specificity of these isoforms overlaps (Radominska-Pandya et al., 1999). Therefore, kinetic analyses of troglitazone glucuronidation in recombinant UGT1A1 and UGT1A10 (Supersomes) were performed. As shown in Fig. 2A, the kinetics of troglitazone glucuronidation in recombinant UGT1A1 at 6 to 200 μM troglitazone fitted to the Michaelis-Menten kinetics. However, when the troglitazone concentration exceeded 200 μM, the troglitazone glucuronosyltransferase activity gradually decreased. A possible explanation for this phenomenon is substrate inhibition or metabolite inhibition. When the apparent enzyme kinetic parameters were estimated by fitting to the Michaelis-Menten eq. 1 with the initial velocity values at 6 to 200 μM troglitazone, the K m value was 58.3 ± 29.2 μM and V max was 12.3 ± 2.5 pmol/min/mg protein (Table 1). When the apparent enzyme kinetic parameters were estimated by fitting to the substrate inhibition eq. 2 with the velocity values at 6 μM to 1 mM troglitazone, the K m value was 141.1 ± 85.8 μM and V max value was 23.8 ± 9.9 pmol/ min/mg protein. As shown in Fig. 2B, the kinetics of troglitazone glucuronidation in recombinant UGT1A10 at 6 to 200 μM troglitazone fitted to the Michaelis-Menten kinetics. However, when the troglitazone concentration exceeded 100 μM, the troglitazone glucuronosyltransferase activity decreased. When the apparent enzyme kinetic parameters were estimated by fitting to the Michaelis-Menten eq. 1 with the initial velocity values at 6 to 200 μM troglitazone, the K m value was 11.1 ± 5.8 μM and V max value was 33.6 ± 3.7 pmol/min/mg protein. When the apparent enzyme kinetic parameters were estimated by fitting to the substrate inhibition eq. 2 with the velocity values at 6 μM to 1 mM troglitazone, the K m value was 497.1 ± 2793.3 μM and V max value was 532.5 ± 2828.1 pmol/ min/mg protein.

Kinetics of Troglitazone Glucuronidation in Human Liver or Jejunum Microsomes. Kinetic analyses of troglitazone glucuronidation in human liver (H161) or jejunum (HJM0040) microsomes were performed. As shown in Fig. 3, the kinetics at 6 to 200 μM troglitazone fitted to the Michaelis-Menten kinetics. However, when the troglitazone concentration exceeded 200 μM, the troglitazone glucuronosyltransferase activity gradually decreased. In human jejunum microsomes, troglitazone glucuronidation was not detected at 750 μM and 1 mM troglitazone (Fig. 3B). When the apparent enzyme kinetic parameters were estimated by fitting to the Michaelis-Menten eq. 1...
with the initial velocity values at 6 to 200 µM troglitazone, the $K_m$ values in human liver and jejunum microsomes were 13.5 ± 2.0 and 8.1 ± 0.3 µM and $V_{max}$ values were 34.8 ± 1.2 and 700.9 ± 4.3 pmol/min/mg protein, respectively (Table 1). When the apparent enzyme kinetic parameters were estimated by fitting to the substrate inhibition eq. 2 with the velocity values at 6 µM to 1 mM troglitazone, the $K_m$ values in human liver and jejunum microsomes were 49.4 ± 26.3 and 62.7 ± 122.8 µM and $V_{max}$ values were 70.2 ± 22.2 and 2138.5 ± 2990.4 pmol/min/mg protein, respectively.

**Metabolite Inhibition of Troglitazone Glucuronosyltransferase Activity in Human Liver Microsomes.** The inhibitory effects of troglitazone glucuronide per se on the troglitazone glucuronosyltransferase activity in the pooled human liver microsomes were determined. Troglitazone glucuronide strongly inhibited the troglitazone
glucuronosyltransferase activity in human liver microsomes (Fig. 4),
giving the IC$_{50}$ value of 0.5 nM. We confirmed that the incubation of
troglitazone glucuronide with the human liver microsomes and UDP-
glucuronic acid, but without troglitazone did not affect the concen-
tration of the added troglitazone glucuronide under the present exper-
imental condition, suggesting that there was no further metabolism of
troglitazone glucuronide.

**Interindividual Variability of Troglitazone Glucuronosyltrans-
ferase Activity from 13 Human Livers and Correlation Analyses.**
Troglitazone glucuronosyltransferase activities in microsomes from
13 human livers were determined at 100 nM troglitazone (Fig. 5A).
The interindividual difference in troglitazone glucuronosyltransferase
activity was at most 2.2-fold (12.0 ± 1.7–27.1 ± 2.3 pmol/min/mg
protein). Correlation analyses were performed between the troglita-
zone glucuronosyltransferase activity and β-estradiol (UGT1A1), bil-
irubin (UGT1A1), trifluoperazine (UGT1A4), or propofol glucuronos-
yltransferase activities provided by the manufacturer, or
4-nitrophenol (UGT1A6), 1-naphthol (UGT1A1, UGT1A6, UGT1A8,
and UGT1A9), or morphine (UGT2B7) glucuronosyltransferase ac-
tivities determined in the present study. The troglitazone glucurono-
syltransferase activities in the 13 human liver microsomes were sig-
nificantly ($r = 0.579$, $p < 0.05$) correlated with the β-estradiol
3-glucuronosyltransferase activities (Fig. 5B). However, the troglita-
zone glucuronosyltransferase did not correlated with the bilirubin
glucuronosyltransferase activities ($r = 0.487$, $p = 0.154$), whereas the
bilirubin glucuronosyltransferase activities were highly correlated
with the β-estradiol 3-glucuronosyltransferase activities ($r = 0.927,$

**TABLE 1**

<table>
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<tr>
<th>Microsomes</th>
<th>Eq. 1 (Hyperbola)$^a$</th>
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<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$V_{max}$ (pmol/min/mg)</td>
<td>$V_{max}/K_m$</td>
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<td>Recombinant UGT1A1</td>
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<tr>
<td>Recombinant UGT1A10</td>
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<td>Human liver microsomes (H161)</td>
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<td>34.8 ± 1.2</td>
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<tr>
<td>Human jejunum microsomes (HJM0040)</td>
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<td>700.9 ± 4.3</td>
<td>86.53</td>
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</tr>
</tbody>
</table>

|                           | Eq. 2 (Substrate Inhibition)$^b$ |                      |                      |
|                           | $K_m$ (µM) | $V_{max}$ (µmol/min) | $V_{max}/K_m$ | $K_i$ (µM) |
| Recombinant UGT1A1        | 141.1 ± 88.5 | 23.8 ± 9.9            | 0.17                | 220.4 ± 130.9 |
| Recombinant UGT1A10       | 497.1 ± 2973.3 | 352.5 ± 2828.1        | 1.07                | 9.8 ± 54.7   |
| Human liver microsomes (H161) | 49.4 ± 26.3 | 70.2 ± 22.2           | 1.52                | 153.7 ± 76.3 |

$^a$ Kinetic parameters were calculated by the Michaelis-Menten equation (eq. 1) with the activity at 6–200 µM substrate concentrations.

$^b$ Kinetic parameters were calculated by the substrate inhibition equation (eq. 2) with the activity at 6 µM–1 mM substrate concentrations.

**FIG. 3.** Kinetics of troglitazone glucuronidation in human liver (H161) (A) or jejunum (HJM0040) (B) microsomes.
The solid line represents the fitting curve to the Michaelis-Menten eq. 1 (6 µM-200 µM, closed circles). The dotted line represents the fitting curve to the substrate inhibition eq. 2 (6 µM–1 mM, closed and open circles). The troglitazone glucuronosyltransferase activity was determined as described under Materials and Methods. Each data point represents the mean of duplicate determinations.

**FIG. 4.** Metabolite (troglitazone glucuronide) inhibition of troglitazone glucuronosyltransferase activity in human liver microsomes.
Troglitazone glucuronide was added to the incubation mixture as an inhibitor. Troglitazone glucuronosyltransferase activity in pooled human liver microsomes (H161) was determined at 100 µM as described under Materials and Methods. Each data point represents the mean of duplicate determinations. The troglitazone glucuronosyltransferase activity in the pooled human liver microsomes in the absence of the inhibitor was 34.2 pmol/min/mg protein.
p < 0.001). In addition, the troglitazone glucuronosyltransferase activities did not correlate with the trifluoperazine (r = 0.310, p = 0.302), propofol (r = 0.453, p = 0.120), 4-nitrophenol (r = 0.306, p = 0.361), 1-naphthol (r = 0.172, p = 0.613), or morphine (r = 0.427, p = 0.160) glucuronosyltransferase activities (data not shown).

Troglitazone Glucuronosyltransferase Activity in Human Intestinal S-9 Sample. Troglitazone glucuronosyltransferase activities in S-9 samples from five human intestines were determined (Fig. 6). The interindividual difference in the troglitazone glucuronosyltransferase activity was about 8.2-fold (0.5–4.1 pmol/min/mg protein).

Inhibition Analyses of Troglitazone Glucuronidation in Human Liver or Jejunum Microsomes. The inhibitory effects of bilirubin (UGT1A1), β-estradiol (UGT1A1 and UGT1A9), 4-nitrophenol (UGT1A6 and UGT1A9), imipramine (UGT1A3 and UGT1A4), emodin (UGT1A8 and UGT1A10), and morphine (UGT2B7) on the troglitazone glucuronosyltransferase activity in human liver or jejunum microsomes were determined. As shown in Fig. 7A, the troglitazone glucuronosyltransferase activity in the pooled human liver microsomes was prominently inhibited by bilirubin (IC50 = 19.0 μM). In contrast, the inhibitory effects of emodin (IC50 = 134.0 μM), β-estradiol (IC50 = 261.2 μM), 4-nitrophenol (IC50 = 379.2 μM), imipramine (IC50 > 500 μM), and morphine (IC50 > 500 μM) were weak. As shown in Fig. 7B, the activity in human jejunum microsomes was prominently inhibited by emodin (IC50 = 13.7 μM). The inhibitory effects of bilirubin (IC50 = 154.0 μM), β-estradiol (IC50 = 361.8 μM), 4-nitrophenol (IC50 = 486.5 μM), imipramine (IC50 > 500 μM), and morphine (IC50 > 500 μM) were weak.

Discussion

All UGT isoforms expressed in human liver, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15, exhibited troglitazone glucuronosyltransferase activity. With immunoblot analysis, relative expression levels of recombinant UGT1A isoforms were determined. Microsomes from UGT1A3 Supersomes, UGT1A3, UGT1A6, and UGT1A10 Baculosomes showed weak band in comparison with the other UGT1A isoforms. It might be due to low expression level or low reactivity of the antibodies to the expression systems. In Supersomes, high troglitazone glucuronosyltransferase activities in UGT1A8 and UGT1A10 were not due to the differences in the expression levels. In Baculosomes, UGT1A10 exhibited high troglitazone glucuronosyltransferase activity in spite of weak immunoreactive band. The troglitazone glucuronosyltransferase activity in human liver microsomes was significantly correlated only with the β-estradiol 3-glucuronosyltransferase activity that was mainly catalyzed by UGT1A1. Furthermore, the activity in human liver microsomes was strongly inhibited by bilirubin, a specific substrate for UGT1A1. These results suggest that the troglitazone glucuronidation in human liver microsomes might be mainly catalyzed by UGT1A1. It has been reported that in Gunn rats, which are hereditarily deficient...
in the UGT1 family, the metabolic profile of troglitazone was much the same as that of Wistar rats (Watanabe et al., 2000), suggesting no contribution of UGT1 family to troglitazone glucuronidation. Concerning the contradiction with our results, the possibility is considered that other UGT isoforms might compensate for UGT1 family in Gunn rats or that the UGT isoforms in rats which are responsible for troglitazone glucuronidation might differ from those in humans as Yoshigae et al. (2000) have also reported.

To our knowledge, this is the first study to show that UGT1A8 and UGT1A10 have high troglitazone glucuronosyltransferase activity. Because UGT1A8 and UGT1A10 are not expressed in liver, these isoforms could be responsible for the extrahepatic metabolism of troglitazone particularly in the gastrointestinal tract. As shown in Fig. 7, the troglitazone glucuronosyltransferase activity in human jejunum microsomes was prominently inhibited by emodin, a typical substrate of UGT1 family, the metabolic profile of troglitazone glucuronidation in human intestines might be higher than that in human livers. When we have examined the imipramine-N-glucuronosyltransferase activity (UGT1A4) in the same lot of human jejunum microsomes (HJM0040), the imipramine-N-glucuronide was not detected, although the activity in human liver microsomes was detectable (Nakajima et al., 2002). Therefore, it would be a characteristic of troglitazone glucuronidation that the activity or clearance in human intestines is higher than that in human liver microsomes. To confirm the higher contribution of intestines than that of livers to the troglitazone glucuronidation, the estimation using several
samples of human intestine microsomes would be needed. Because the microsomes from the human livers and intestine were obtained from different manufacturers, these were inevitably from different donors. The use of the human liver and intestine microsomes from the same donors is desirable to estimate the contribution to the clearance of drugs.

Troglitazone sulfate and glucuronide are excreted into bile. Both metabolites are converted back to the parent compound, troglitazone, in the small intestine by arylsulfotransferase and β-glucuronidase. In the enterohepatic circulation troglitazone would be converted to glucuronide mainly by UGT1A1 in human livers. Furthermore, in enterocytes, it may also be converted to glucuronide by UGTs such as UGT1A8 and UGT1A10. Using knockout rats of multidrug-resistant associated protein-2 (Mrp-2), it has been demonstrated that troglitazone glucuronide is a substrate of Mrp2 (Kostrubsky et al., 2001). Therefore, the troglitazone glucuronide formed in enterocytes might be excreted to the intestinal lumen via transporters such as Mrp2 expressed in the brush-border membrane. Then, the glucuronide would again be converted to troglitazone by β-glucuronidase and the troglitazone might be reabsorbed.

In conclusion, it was demonstrated that the clearance of troglitazone glucuronidation in human intestines is higher than that in human livers. UGT1A8 and UGT1A10 as well as UGT1A1 would significantly contribute to the troglitazone glucuronidation in human intestines.

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References


