Glucuronidations of endobiotics and xenobiotics are catalyzed by UDP-glucuronosyltransferase (UGT) (Miners and Mackenzie, 1991). It is well known that there are many isoforms of mammalian UGT enzymes (Tukey and Strassburg, 2000). To date, three UGT families have been identified in humans: UGT1, UGT2, and UGT3. Of these three families, UGT1 and UGT2 have been shown to catalyze the glucuronidation of xenobiotics. The UGT1 and UGT2 genes appear to be structurally different in that the UGT1 proteins result from alternate splicing of different first exons with five shared exons encoded by the UGT1 gene complex, whereas UGT2 proteins appear to be encoded by unique genes. In the human genome, at least 13 different first exons have been identified for the UGT1 gene (Gong et al., 2001). Five of these human UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9, are expressed in the liver (Strassburg et al., 1999, 2000). UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A10 are expressed in human intestine (Strassburg et al., 2000; Tukey and Strassburg, 2000). UGT1A1 has been shown to catalyze the glucuronidation of bilirubin, phenolic compounds, certain estrogens, oripavine opioids, and coumarins (Bosma et al., 2000; Tukey and Strassburg, 2000). UGT1A3 and UGT1A4 catalyze N-glucuronidation of primary, secondary, and tertiary amines (Green and Tephly, 1996; Green et al., 1998). In addition, UGT1A3 and UGT1A4 can also catalyze the O-glucuronidation of opioids, coumarins, and phenols (Green et al., 1998). UGT1A6 preferentially catalyzes the glucuronidation of planar phenols, whereas UGT1A9 catalyzes the glucuronidation of bulky phenols such as propofol, flavonoids, and certain aliphatic alcohols (Ebner and Burchell, 1993). UGT1A6 and UGT1A9 have been shown to catalyze the glucuronidation of primary and secondary amines (Huskey et al., 1994; Orzechowski et al., 1994). Although specific substrates have begun to be used to elucidate the pattern of gene expression of these isoforms in different tissues (Radominska-Pandya et al., 1999), standard conditions to assess in vitro glucuronidation have not been established yet.

Imipramine is known to be metabolized to imipramine N-glucuronide (Green et al., 1995). The percentage of the dose excreted in urine as imipramine N-glucuronide was reported to be 0.2% (Luo et al., 1995). Until now, imipramine N-glucuronide formed by in vitro assay

**ABSTRACT:**
A method for the direct determination of imipramine N-glucuronidation in human liver microsomes by high-performance liquid chromatography with UV detection was developed. Imipramine was incubated with human liver microsomes and UDP-glucuronic acid. The Eadie-Hofstee plots of imipramine N-glucuronidation in human liver microsomes were biphasic. For the high-affinity component, the $K_m$ was 97.2 ± 39.4 μM and the $V_{max}$ was 0.29 ± 0.03 nmol/min/mg of protein. For the low-affinity component, the $K_m$ was 0.70 ± 0.29 mM and the $V_{max}$ was 0.90 ± 0.28 nmol/min/mg of protein. The imipramine N-glucuronosyltransferase activities were not detectable in two samples of human jejunum microsomes. Among recombinant UDP-glucuronosyltransferases (UGTs) in baculovirus-infected insect cells (Supersomes or Baculosomes) or human B-lymphoblastoid cells tested in the present study (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15), only UGT1A4 showed imipramine N-glucuronosyltransferase activity. The activity in UGT1A4 Supersomes was higher than that in recombinant UGT1A4 expressed in human B-lymphoblastoid cells at all imipramine concentration tested. The kinetics of imipramine N-glucuronidation in UGT1A4 Supersomes did not fit the Michaelis-Menten plot, showing a $K_m$ of > 1 mM. In contrast, in UGT1A4 expressed in human B-lymphoblastoid cells, $K_m$ was 0.71 ± 0.36 mM and the $V_{max}$ was 0.11 ± 0.03 nmol/min/mg of protein. Interindividual differences in the imipramine N-glucuronidation in liver microsomes from 14 humans were at most 2.5-fold. The imipramine N-glucuronosyltransferase activities in 11 human liver microsomes were significantly ($r = 0.817, P < 0.005$) correlated with the glucuronosyltransferase activities of trifluoperazine, a typical substrate of UGT1A4. This is the first report of the biphasic kinetics of imipramine N-glucuronide in human liver microsomes.

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had been determined by TLC with 14C-labeled UDP-glucuronic acid (Green et al., 1995, 1998) or by radio-HPLC with 3H-labeled imipramine (Coughtrie and Sharp, 1991). Therefore, one of the purposes of the present study was to develop a simple HPLC-UV method for directly determining imipramine N-glucuronidation in vitro.

Another purpose was to characterize thoroughly imipramine N-glucuronidation in human liver microsomes. Until now, no kinetic analysis of imipramine N-glucuronidation in human liver microsomes has been performed. Therefore, in the present study, the kinetics of imipramine N-glucuronidation in human liver microsomes were investigated. Furthermore, imipramine N-glucuronosyltransferase activities in human jejunum microsomes and all recombinant UGT isoforms now commercially available were also determined.

**Experimental Procedures**

**Materials.** Imipramine hydrochloride was purchased from Wako Pure Chemicals (Osaka, Japan). UDP-glucuronic acid and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO). Pooled human liver microsomes (H161) and microsomes from 14 individual human livers (H003, H006, H023, H030, H042, H043, H056, H066, H070, H089, H093, H112, HK23, and HK34) were purchased from Gentest Corp. (Woburn, MA). Glucuronosyltransferase activities of estradiol, trifluoperazine, and propofol as typical substrates of UGT1A1, UGT1A4, and UGT1A9, respectively, in these human liver microsomes except for H006, H030, and H070 were provided by the manufacturer. The human jejunum microsomes (HJM0023 and HJM0040) were obtained from KAC (Shiga, Japan). Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15 expressed in baculovirus-infected insect cells (Supersomes) were from Gentest Corp. Recombinant UGT1A1, UGT1A4, UGT1A6, and UGT1A9 expressed in human B-lymphoblastoid cells were also from Gentest Corp. Recombinant UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A10, and UGT2B7 expressed in baculovirus-infected insect cells (Baculosomes) were from PanVera Corp. (Madison, WI). All other chemicals and solvents were of the highest grade commercially available.

**Imipramine N-Glucuronidation Assay.** A typical incubation mixture (200 μl total volume) contained 50 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl2, 5 mM UDP-glucuronic acid, 25 μg/ml alamethicin, 0.25 mg/ml human liver microsomes (0.25 mg/ml human jejunum microsomes or 0.5 mg/ml for recombinant UGT), and 0.5 mM imipramine. The reactions were initiated by the addition of UDP-glucuronic acid, and the reaction mixtures were incubated for 60 min. The reactions were then terminated by boiling for 10 min. After removal of the protein by centrifugation at 10,000 rpm for 5 min, a 20-μl portion of the sample was subjected to HPLC. Chromatography was performed using an L-6000 pump (Hitachi, Tokyo, Japan), an L-4200 UV-VIS detector (Hitachi), an AS-8010 autosampler (Tosoh, Tokyo, Japan), an 865-CO column oven (Jasco), and a Mighty-Vue recorder (Jasco, Tokyo, Japan), with a Symmetry C18 column (2.1 × 150 mm; 5 μm; Waters Corp., Milford, MA). The flow rate was 0.25 ml/min, and the column temperature was 40°C. The mobile phases were 35% CH3CN, 50 mM KH2PO4 (pH 5.0), and 50 mM KH2PO4 (pH 7.4), 5 mM MgCl2, 5 mM UDP-glucuronic acid, 25 μg/ml alamethicin, 0.25 mg/ml human liver microsomes (0.25 mg/ml human jejunum microsomes or 0.5 mg/ml for recombinant UGT), and 0.5 mM imipramine. The reactions were initiated by the addition of UDP-glucuronic acid, and the reaction mixtures were incubated for 60 min. The reactions were then terminated by boiling for 10 min. After removal of the protein by centrifugation at 10,000 rpm for 5 min, a 20-μl portion of the sample was subjected to HPLC. Chromatography was performed using an L-6000 pump (Hitachi, Tokyo, Japan), an L-4200 UV-VIS detector (Hitachi), an AS-8010 autosampler (Tosoh, Tokyo, Japan), an 807-IT integrator (Jasco, Tokyo, Japan), and an 865-CO column oven (Jasco) with a Mighty-Vue recorder (Jasco, Tokyo, Japan). The flow rate was 0.5 ml/min and the column temperature was 35°C. The eluent was monitored at 205 nm. The mobile phases were 35% CH3CN, 50 mM KH2PO4 (pH 7.4), and 50 mM KH2PO4 (pH 5.0).

For the quantification of imipramine N-glucuronide, the eluate of the HPLC from the incubation mixture with human liver microsomes including imipramine N-glucuronide was collected. A part of the eluate was hydrolyzed with NaOH at 75°C for 30 min (Hawes, 1998). The completely hydrolyzed imipramine N-glucuronide was quantified as imipramine by HPLC. Once we determined the peak height per known content of imipramine N-glucuronide, the ratio was applied to the calculation of the imipramine N-glucuronide formed in the incubation mixtures.

**Identification of Imipramine N-Glucuronide by LC-MS/MS Analysis.** LC-MS/MS analysis was performed using a LCQDeca (ThermoQuest, San Jose, CA) under electrospray ionization (ESI) conditions. The operation conditions used were as follows: capillary temperature, 350°C; capillary volt, 3 V; tube lens volt, −45 V; sheath gas, N2; pressure, 90 psi; auxiliary gas, N2; 20 l/min; and collision energy, 40%. LC was performed using an HP1100 (Agilent Technologies, Palo Alto, CA) with a Symmetry C18 column (2.1 × 150 mm; 5 μm; Waters Corp., Milford, MA). The flow rate was 0.25 ml/min, and the column temperature was 40°C. The mobile phases were 10 mM AcONH4 (A) and CH3CN (B). Typical conditions for elution were as follows: 30 to 70% B (0–5 min) and 70% B (5–10 min). The retention times of imipramine N-glucuronide and imipramine were 3.4 and 8.5 min, respectively.

**Kinetic Analyses.** The kinetic studies were performed using human liver microsomes and recombinant human UGT1A4 expressed in baculovirus-infected insect cells (Supersomes) or human B-lymphoblastoid cells. In determining the kinetic parameters, the imipramine concentration ranged 10 μM–2 mM. Eadie-Hofstee plots were constructed to determine whether the kinetics were mono- or biphasic. Kinetic parameters were estimated from the fitted curves using a computer program of Kaleidagraph (Synergy Software, Reading, PA) designed for nonlinear regression analysis.

**Correlation Analyses.** Correlation analyses between imipramine N-glucuronidation and estradiol (UGT1A1), trifluoperazine (UGT1A4), and propofol (UGT1A9) glucuronosyltransferase activities were determined by Pearson’s product-moment method. A P value of less than 0.05 was considered statistically significant.

**Results**

**Chromatography.** Figure 1 shows representative chromatograms of the imipramine N-glucuronide formation in the pooled human liver microsomes. Figure 1, A and B, represents chromatograms of the incubation mixture for the imipramine N-glucuronidation without and with UDP-glucuronic acid, respectively. The retention times of imipramine N-glucuronide and imipramine were 6.2 and 16.8 min, respectively.
None of these chromatograms showed any interfering peaks with imipramine N-glucuronide. It was confirmed that the peak of imipramine N-glucuronide disappeared after alkaline hydrolysis (data not shown).

**LC-MS/MS Analyses of Imipramine N-Glucuronide.** The ESI mass spectrum of a peak typically formed by incubation of imipramine with human liver microsomes is shown in Fig. 2A. [M + H]$^+$ ion at m/z 457 corresponding to imipramine N-glucuronide was observed. The ESI mass spectrum of a peak formed by incubation of imipramine with human liver microsomes and UDP-glucuronic acid, and ESI mass spectrum of imipramine are indicated.

The [M + H]$^+$ ion at m/z 457 corresponds to imipramine N-glucuronide (A). The product ion spectrum showed a peak at m/z 281 corresponding to the protonated imipramine (B). The fragment ions of m/z 236, m/z 208, and m/z 193 (B) are the same as those of imipramine (C). The fragmentation patterns of imipramine N-glucuronide and imipramine are indicated.

**FIG. 2.** ESI mass spectrum (A) and product ion spectrum (B) of the peak formed by the incubation of imipramine with human liver microsomes and UDP-glucuronic acid, and ESI mass spectrum (C) of imipramine.
served. The product ion spectrum of the peak showed that a loss of the glucuronic acid element (176 atomic mass units) yields the protonated aglycon ion at m/z 281 (Fig. 2B). The fragment ions at m/z 236, m/z 208, and m/z 193 (Fig. 2B) were the same as those of imipramine (Fig. 2C). From these observations, it was confirmed that the peak formed by the incubation of imipramine with human liver microsomes, and UDP-glucuronic acid (Fig. 2A) was imipramine N-glucuronide.

Imipramine N-Glucuronidation in Human Liver Microsomes or Human Jejunum Microsomes. Figure 3 shows the imipramine N-glucuronidation in pooled human liver microsomes. The formations increased in a microsomal protein concentration- and time-dependent manner. The formation was linear at least at 0.5 mg/ml of microsomal protein and 120 min incubation. The imipramine N-glucuronidation was dependent on the concentration of UDP-glucuronic acid. The imipramine N-glucuronosyltransferase activity in the pooled human liver microsomes was 1.8 nmol/min/mg of protein. The imipramine N-glucuronosyltransferase activity was not detectable in two samples of human jejunum microsomes (HJM0023 and HJM0040).

Imipramine N-Glucuronidation in Recombinant UGT. All recombinant UGT isoforms expressed in human B-lymphoblastoid cells or baculovirus-infected insect cells (Supersomes or Baculosome), which are commercially available, were used to determine their imipramine N-glucuronosyltransferase activities. Among them, only recombinant UGT1A4 showed significant imipramine N-glucuronosyltransferase activity. The activity in recombinant UGT1A4 expressed in baculovirus-infected insect cells (Supersomes) was higher than that in recombinant UGT1A4 expressed in human B-lymphoblastoid cells (115.9 pmol/min/mg of protein versus 45.0 pmol/min/mg of protein). These activities were considerably lower than those in human liver microsomes at 0.5 mM imipramine.

Kinetics of Imipramine N-Glucuronidation in Human Liver Microsomes and Recombinant UGT1A4. Kinetic analyses of imipramine N-glucuronidation in human liver microsomes were performed. As shown in Fig. 4B, the Eadie-Hofstee plot for imipramine N-glucuronidation in human liver microsomes was biphasic, indicating that multiple enzymes are responsible for the bioransformation. For the high-affinity component in the microsomes, the $K_m$ was 97.2 ± 39.4 µM and the $V_{max}$ was 0.29 ± 0.03 nmol/min/mg of protein in human microsomes from four livers (Table 1). For the low-affinity component in the microsomes, the $K_m$ was 0.70 ± 0.29 mM and the $V_{max}$ was 0.90 ± 0.28 nmol/min/mg of protein. The kinetic parameters for imipramine N-glucuronidation in recombinant human UGT1A4 expressed in baculovirus-infected insect cells (Supersomes) or human B-lymphoblastoid cells were also determined. As shown in Fig. 4C, the kinetics in recombinant human UGT1A4 Supersomes did not fit the Michaelis-Menten plot, showing a $K_m$ of >1 mM. In contrast, in UGT1A4 expressed in human B-lymphoblastoid cells, $K_m$ was 0.71 ± 0.36 mM and the $V_{max}$ was 0.11 ± 0.03 nmol/min/mg of protein.

Interindividual Variability in Imipramine N-Glucuronidation in Human Liver Microsomes and Correlation with Trifluoperazine Glucuronosyltransferase Activity. The imipramine N-glucuronosyltransferase activities in microsomes from 14 human livers were determined (Fig. 5). The interindividual difference in imipramine N-glucuronidation was at most 2.5-fold (0.29–0.72 nmol/min/mg of protein, 0.47 ± 0.13 nmol/min/mg of protein). The imipramine N-glucuronosyltransferase activities in the 11 human liver microsomes were significantly ($r = 0.817, P < 0.005$) correlated with the trifluoperazine glucuronosyltransferase activities (Fig. 6). In contrast, no significant correlation was observed with the estradiol glucuronosyltransferase activities ($r = 0.182, P = 0.591$) or propofol glucuronosyltransferase activities ($r = 0.408, P = 0.213$).

Discussion

We developed a simple HPLC method for the in vitro determination of imipramine N-glucuronidate. It was confirmed that the peak formed by the incubation of imipramine with human liver microsomes and UDP-glucuronic acid was imipramine N-glucuronidate by the effects of alkaline hydrolysis and the LC-MS/MS analyses. The MS and MS/MS spectra of the peak profiles were consistent with the MS spectrum of imipramine N-glucuronidate in a previous report of Lehman et al. (1983). It has been reported that the determination of imipramine N-glucuronidate in human urine was difficult because it coeluted with hydroxydesmethylimipramine glucuronidate in the TLC and anion exchange systems (Lehman et al., 1983). Although it was not investigated in the present study, the present HPLC method could possibly be applied to determine the imipramine N-glucuronidate in human plasma or urine. Such a study will be performed in the near future.

Eadie-Hofstee plots of imipramine N-glucuronidation in human liver microsomes were clearly biphasic, indicating the involvement of multiple enzymes. However, among the recombinant UGT isoforms tested in the present study, only UGT1A4 showed imipramine N-glucuronidation. Furthermore, the imipramine N-glucuronosyltransferase activities in the 11 human liver microsomes were significantly correlated only with the trifluoperazine glucuronosyltransferase activities that are recognized to be catalyzed by UGT1A4. These results suggest that a major enzyme that catalyzes imipramine N-glucuronidation in human liver microsomes would be UGT1A4. It must be noted that the $K_m$ value of the imipramine N-glucuronidation in recombinant UGT1A4 expressed in human B-lymphoblastoid cells was near to those of low affinity component in human liver microsomes. In contrast, the $K_m$ value in recombinant UGT1A4 Super-

Fig. 3. Formations of imipramine N-glucuronidate as a function of the protein concentration of human liver microsomes (A), incubation time (B), and the concentration of UDP-glucuronic acid (C).

Unless specified, the standard incubation mixture contained 0.25 mg/ml of microsomal protein, 0.5 mM imipramine, and 5 mM UDP-glucuronic acid, and was incubated at 37°C for 60 min. Each data point represents the mean of duplicate determinations. The imipramine N-glucuronosyltransferase activity in the pooled human liver microsomes was 1.8 nmol/min/mg of protein.
somes was higher than those of low affinity component in human liver microsomes. Taking this information into consideration, whether UGT1A4 is the only enzyme that catalyzes imipramine N-glucuronidation in human liver microsomes, or whether UGT1A4 is either a high or low affinity component of the imipramine N-glucuronidation, cannot be determined on the basis of the present data. It has been reported that, among many UGT isoforms, only UGT1A3 and UGT1A4 catalyze the glucuronidation of tertiary amine compounds to quaternary ammonium-linked glucuronides (Green et al., 1995; Green and Tephly, 1996, 1998; Breyer-Pfaff et al., 2000). It has also been reported that the glucuronosyltransferase activities of recombinant UGT1A3 were lower than those of recombinant UGT1A4 for several substrates such as 2-aminobiphenyl, cyproheptadine, and amitriptyline (Green et al., 1998). Thus, it was suspected that the activity of the recombinant UGT1A3 in the expression system was possibly too low to show imipramine N-glucuronidation. In the present study, the contribution of UGT1A3 to the imipramine N-glucuronidation could not be proven. It has been reported that the kinetics of amitriptyline and diphenhydramine N-glucuronidations, which are also considered to be catalyzed by UGT1A4, were biphasic in human liver microsomes (Breyer-Pfaff et al., 1997). The cause of the biphasic kinetics also remains to be solved.

**TABLE 1**

<table>
<thead>
<tr>
<th>Human Liver Microsomes</th>
<th>High-Affinity Component</th>
<th>Low-Affinity Component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_M$ (μM)</td>
<td>$V_{max}$ (nmol/min/mg protein)</td>
</tr>
<tr>
<td>H003</td>
<td>78.9</td>
<td>0.33</td>
</tr>
<tr>
<td>H030</td>
<td>75.6</td>
<td>0.26</td>
</tr>
<tr>
<td>H042</td>
<td>103.2</td>
<td>0.26</td>
</tr>
<tr>
<td>H112</td>
<td>149.2</td>
<td>0.32</td>
</tr>
<tr>
<td>mean ± S.D.</td>
<td>97.2 ± 39.4</td>
<td>0.29 ± 0.03</td>
</tr>
</tbody>
</table>
A prominent difference in the imipramine N-glucuronosyltransferase activities by recombinant UGT1A4 expressed in baculovirus-infected insect cells and in those human B-lymphoblastoid cells was observed. The kinetic properties were also different between the two expression systems (\(K_m > 1 \text{mM in UGT1A4 Supersomes versus } K_m = 0.71 \pm 0.36 \text{mM in UGT1A4 expressed in human B-lymphoblastoid cells}\)). One of the factors of the difference would be the difference in the expression level of UGT1A4 between the two expression systems. Another factor might be a difference in the membrane circumstances in the expression systems. Indeed, it has been reported that the nature of the phospholipid environment influences the rate-limiting step of glucuronidation (Magdalou et al., 1982). Similar differences in the activities between the two expression systems were already shown for cytochrome P450 (Nakajima et al., 1999) and were considered to be due to differences in the membrane circumstances. Previously, Green et al. (1998) constructed an expression system of UGT1A3 and UGT1A4 in human embryonic kidney 293 (HK293) cells and determined the kinetic parameters of imipramine N-glucuronidation. They reported that the \(K_m\) value was 514 \(\mu\text{M}\) and the \(V_{\text{max}}\) value was 8 pmol/min/mg of protein for recombinant UGT1A3, and the \(K_m\) value was 310 \(\mu\text{M}\) and the \(V_{\text{max}}\) value was 180 pmol/min/mg of protein for recombinant UGT1A4. In that study, imipramine N-glucuronosyltransferase activities were determined by TLC with 2 mM \([^{14}\text{C}]\) UDP-glucuronic acid under the condition of pH 8.4. Thus, the difference between the kinetic parameters of imipramine N-glucuronidation in recombinant UGT1A4 in our study and those in their study might be due to differences in the experimental conditions and/or the membrane circumstances of the host cells.

Trifluoperazine is a tertiary amine that has been shown to be metabolized to glucuronide by recombinant UGT1A4 expressed in HK293 cells (Green and Tephly, 1996). With recombinant UGT1A4, imipramine and trifluoperazine glucuronosyltransferase activities were reported to be 110 \(\pm\) 11 pmol/min/mg of protein and 165 \(\pm\) 18 pmol/min/mg of protein, respectively, at a substrate concentration of 0.5 mM (Green and Tephly, 1996). The human UGT1A4 (Supersomes) used in the present study was reported by the manufacturer to have trifluoperazine glucuronosyltransferase activity of 1,450 pmol/min/mg of protein at a substrate concentration of 0.2 mM. However, the imipramine N-glucuronosyltransferase activity in the UGT1A4 (Supersomes) was 115.9 pmol/min/mg of protein at 0.5 mM imipramine in the present study. The cause of the discrepancy in the potencies of glucuronidation for imipramine and trifluoperazine between the previous report (Green and Tephly, 1996) and the present study is unknown. The differences in the experimental conditions and/or the membrane circumstances of the host cells might also be the cause of this difference. Unfortunately, whether UGT isoforms other than UGT1A4 can catalyze the trifluoperazine glucuronidation has never been investigated.

It has been reported that the imipramine N-glucuronidation in human intestine microsomes was higher than that in human liver microsomes (Strassburg et al., 2000). However, such activities in microsomes from two samples of human jejunum were never detected in the present study. We confirmed that the two samples of human jejunum microsomes were active for the glucuronidation of the other substrate, troglitazone. Strassburg et al. (2000) reported that the expressions of UGT1A1, UGT1A3, UGT1A4, and UGT1A6 were polymorphic in intestine. Therefore, the discrepancy might be due to the polymorphic expression of the UGT1A isoforms in human intestine.

In conclusion, the biphasic kinetics of imipramine N-glucuronidation in human liver microsomes were first demonstrated in the present study. It was also shown that one of the enzymes involved in the imipramine N-glucuronidation in humans is UGT1A4.

Acknowledgments. We acknowledge Brent Bell for reviewing the manuscript.

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