TRANS-3’-HYDROXYCOTININE O- AND N-GLUCURONIDATIONS IN HUMAN LIVER MICROSONES

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ABSTRACT:

Trans-3’-hydroxycotinine is a major metabolite of nicotine in humans and is mainly excreted as O-glucuronide in smoker’s urine. Incubation of human liver microsomes with UDP-glucuronic acid produces not only trans-3’-hydroxycotinine O-glucuronide but also N-glucuronide. The formation of N-glucuronide exceeds the formation of O-glucuronide in most human liver microsomes, although N-glucuronide has never been detected in human urine. Trans-3’-hydroxycotinine N-glucuronidation in human liver microsomes was significantly correlated with nicotine and cotinine N-glucuronidations, which are catalyzed mainly by UDP-glucuronosyltransferase (UGT1A4) and was inhibited by imipramine and nicotine, which are substrates of UGT1A4. Recombinant UGT1A4 exhibited substantial trans-3’-hydroxycotinine N-glucuronosyltransferase activity. These results suggest that trans-3’-hydroxycotinine O-glucuronidation in human liver microsomes would be mainly catalyzed by UGT1A4. In the present study, trans-3’-hydroxycotinine O-glucuronidation in human liver microsomes was thoroughly characterized, since trans-3’-hydroxycotinine O-glucuronide is one of the major metabolites of nicotine. The kinetics were fitted to the Michaelis-Menten equation with a \( K_m \) of 10.0 ± 0.8 mM and a \( V_{\text{max}} \) of 85.8 ± 3.8 pmol/min/mg. Among 11 recombinant human UGT isoforms expressed in baculovirus-infected insect cells, UGT2B7 exhibited the highest trans-3’-hydroxycotinine O-glucuronosyltransferase activity (1.1 pmol/min/mg) followed by UGT1A9 (0.3 pmol/min/mg), UGT2B15 (0.2 pmol/min/mg), and UGT2B4 (0.2 pmol/min/mg) at a substrate concentration of 1 mM. Trans-3’-hydroxycotinine O-glucuronosyltransferase activity by recombinant UGT2B7 increased with an increase in the substrate concentration up to 16 mM (10.5 pmol/min/mg). The kinetics by recombinant UGT1A9 were fitted to the Michaelis-Menten equation with a \( K_m \) of 1.6 ± 0.1 mM and \( V_{\text{max}} \) of 0.69 ± 0.02 pmol/min/mg of protein. Trans-3’-hydroxycotinine O-glucuronosyltransferase activities in 13 human liver microsomes ranged from 2.4 to 12.6 pmol/min/mg and were significantly correlated with valproic acid glucuronidation (\( r = 0.716, p < 0.01 \)), which is catalyzed by UGT2B7, UGT1A6, and UGT1A9. Trans-3’-hydroxycotinine O-glucuronosyltransferase activity in human liver microsomes was inhibited by imipramine (a substrate of UGT1A4, \( IC_{50} = 55 \mu M \)), androstaneol (a substrate of UGT2B15, \( IC_{50} = 169 \mu M \)), and propofol (a substrate of UGT1A9, \( IC_{50} = 296 \mu M \)). Interestingly, imipramine (\( IC_{50} = 45 \mu M \)), androstaneol (\( IC_{50} = 21 \mu M \)), and propofol (\( IC_{50} = 41 \mu M \)) also inhibited trans-3’-hydroxycotinine O-glucuronosyltransferase activity by recombinant UGT2B7. These findings suggested that trans-3’-hydroxycotinine O-glucuronidation in human liver microsomes is catalyzed by mainly UGT2B7 and, to a minor extent, by UGT1A9.

Trans-3’-hydroxycotinine O-glucuronide is a major metabolite of nicotine in smoker’s urine. Nicotine is metabolized to cotinine by CYP2A6 (Nakajima et al., 1996a), and cotinine is further metabolized to trans-3’-hydroxycotinine by CYP2A6 (Nakajima et al., 1996b). Glucuronide conjugates of nicotine, cotinine, and trans-3’-hydroxycotinine account for more than 40% of the nicotine dose (Byrd et al., 1992; Benowitz et al., 1994; Yamanaka et al., 2004). Nicotine and cotinine are metabolized to N-glucuronide (3–13 and 8–20% nicotine dose, respectively), whereas trans-3’-hydroxycotinine is metabolized to O-glucuronide (8–13% nicotine dose) in vivo. As far as we know, there is no information about the pharmacological activity of glucuronides of nicotine and its metabolites. However, it is emphasized that a first step of nicotine metabolism to cotinine extinguishes the pharmacological activity of nicotine. In general, glucuronidation is known as a detoxification pathway, since it enhances the elimination of compounds from the body. The theory would be true in glucuronidation of nicotine and its metabolites. Thus, glucuronidation is an important pathway of nicotine metabolism in humans. In our previous study, we clarified that nicotine and cotinine N-glucuronidation are catalyzed mainly by UGT1A4 (Nakajima et al., 2002b), a finding that was subsequently supported by Kuehl and Murphy (2003a). Recently, Kuehl and Murphy (2003b) detected N-linked glucuronide of trans-3’-hydroxycotinine by incubation with human liver microsomes. Nevertheless, the metabolite has never been detected in smoker’s urine (Byrd et al., 1994). Since the trans-3’-hydroxycotinine N-glucu-
tion was significantly correlated with nicotine and cotinine N-glucuronidations, Kuehl and Murphy (2003b) suggested that trans-3'-hydroxycotinine N-glucuronidation may be catalyzed by UGT1A4. Although trans-3'-hydroxyoctine O-glucuronide is the major glucuronide conjugate in smoker’s urine, the UGT isoform(s) catalyzing the O-glucuronidation of trans-3'-hydroxycotinine has never been identified. In the present study, we characterized the trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes and identified the human UGT isoform(s) involved in the glucuronidation compared with trans-3'-hydroxycotinine N-glucuronidation.

Materials and Methods
Materials. Trans-3'-hydroxycotinine was kindly provided by Dr. William S. Caldwell (R. J. Reynolds Tobacco Company, Winston Salem, NC). Trans-3'-hydroxycotinine O- and N-glucuronides were purchased from Toronto Research Chemicals (Ontario, ON, Canada). UDP-glucuronic acid (UDPGA) and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO). Valproic acid and imipramine hydrochloride were purchased from Wako Pure Chemicals (Osaka, Japan). Morphine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). Propofol was kindly supplied by AstraZeneca (London, UK). Pooled human liver microsomes (H161), microsomes from 13 individual human livers (H003, H006, H030, H043, H064, H066, H070, H089, H093, H095, H112, HK23, and HK34), and recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B7, and UGT2B15 expressed in baculovirus-infected insect cells (Supersomes) were purchased from BD Gentest (Woburn, MA). All other chemicals and solvents were of the highest grade commercially available.

Trans-3'-Hydroxycotinine Glucuronidation Assay. A typical incubation mixture (100 µl of total volume) contained 20 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl2, 2.5 mM UDPGA, 25 µg/ml alamethicin, 0.5 mg/ml human liver microsomes or recombinant UGTs, and 1 mM trans-3'-hydroxycotinine. The reactions were initiated by the addition of UDPGA and were then incubated at 37°C for 60 min. The reactions were terminated by 50 µl of ice-cold methanol. After the centrifugation at 12,000 rpm for 5 min, the supernatant was filtered with a 0.22-µm filter (Ultrafree-MC centrifugal filter unit). Aliquots of 5 µl were injected into the LC-MS/MS system. The apparatus was the same as described above except for the column, a Cadenza CD-C18 (2.0 × 150 mm; 3 µm) column (Intakt Corporation, Kyoto, Japan). The mobile phase was 0.01% ammonia (A) and methanol (B), and the conditions for elution were as follows: 0–70% B (0–7 min); 70–0% B (7–11 min). Linear gradients were used for all solvent changes. The flow rate was 0.2 ml/min, and the column temperature was 35°C. The MS/MS was operated in the negative electrospray ionization mode. The turbo gas was maintained at 550°C. Nitrogen was used as the nebulizing, turbo, and curtain gas at 60, 85, and 40 psi, respectively. The collision energy was −10 V and −20 V for valproic acid and valproic acid glucuronide, respectively. Mass/charge ion transitions were recorded in the multiple reaction monitoring mode: m/z 143 and 143 for valproic acid; m/z 151 and 143 for valproic acid glucuronide. The retention times of valproic acid and valproic acid glucuronide were 15.0 and 16.2 min, respectively. For the quantification of valproic acid glucuronide, the eluate of the LC from the incubation mixture with human liver microsomes, including valproic acid glucuronide, was collected referring to the retention time. A part of the eluate was incubated with 800 U/ml β-glucuronidase at 37°C for 24 h. Hydrolyzed valproic acid glucuronide was quantified as valproic acid by LC-MS/MS. Once we determined the peak area per known content of valproic acid glucuronide, the ratio was applied to the calculation of the valproic acid glucuronide formed in the incubation mixtures.

Correlation Analyses. Correlation between trans-3'-hydroxycotinine O- and N-glucuronidations and the other glucuronosyltransferase activities were determined by Pearson’s product-moment method. A p value of less than 0.05 was considered statistically significant.

Inhibition Analyses of Trans-3'-Hydroxycotinine Glucuronidation in Human Liver Microsomes or Recombinant UGT2B7 and UGT1A9. Imipramine, a substrate for UGT1A1 (Nakajima et al., 2002a), propofol, a substrate for UGT1A4 (McGurk et al., 1998; Hanioka et al., 2001), morphine, a substrate for UGT2B7 (Coffman et al., 1997), androstanediol, a substrate for UGT2B15 (Turgeon et al., 2001), and nicotine and cotinine, substrates for UGT1A4 and UGT1A9 (Ghoshen and Hawes, 2002; Nakajima et al., 2002b, Kuehl and Murphy, 2003a) were tested for their inhibitory effects on trans-3'-hydroxycotinine O- and N-glucuronidations in pooled human liver microsomes. The effects of imipramine, propofol, morphine, and androstanediol on the trans-3'-hydroxycotinine O-glucuronidation by recombinant UGT2B7 and the effects of imipramine and propofol on the trans-3'-hydroxycotinine O-glucuronidation by recombinant UGT1A9 were also investigated.

Results

Trans-3'-Hydroxycotinine O- and N-Glucuronidations in Human Liver Microsomes. When the human liver microsomes were incubated with trans-3'-hydroxycotinine and UDPGA, trans-3'-hydroxycotinine O- and N-glucuronides were detected (Fig. 1). The formations of these glucuronides increased in microsomal protein concentration- and time-dependent manners. The formations were linear at least at 1 mg/ml microsomal protein and a 120-min incubation (data not shown). Unless specified, the standard incubation mixture containing 0.5 mg/ml microsomal protein and 2.5 mM UDPGA was incubated at 37°C for 60 min. In our preliminary study, the trans-3'-hydroxycotinine O- and N-glucuronidations in human micro-
somess were not changed at pH 7.4–8.6. The kinetics of trans-3′-hydroxycotinine O- and N-glucuronidations in pooled human liver microsomes were fitted to the Michaelis-Menten equation (Fig. 2, A and B). Eadie-Hofstee plots for both trans-3′-hydroxycotinine O- and N-glucuronidations were linear (Fig. 2, C and D). The apparent $K_m$ and $V_{\text{max}}$ values of trans-3′-hydroxycotinine O-glucuronidation in human liver microsomes were 10.0 ± 0.79 mM and 85.8 ± 3.8 pmol/min/mg of protein, respectively. The apparent $K_m$ and $V_{\text{max}}$ values of trans-3′-hydroxycotinine N-glucuronidation in human liver microsomes were 6.7 ± 1.5 mM and 83.4 ± 8.9 pmol/min/mg of protein, respectively.

Trans-3′-Hydroxycotinine O- and N-Glucuronidations by Recombinant UGT Isoforms. Eleven recombinant UGT isoforms expressed in baculovirus-infected insect cells were used to determine their trans-3′-hydroxycotinine O- and N-glucuronosyltransferase activities. As shown in Fig. 3, UGT2B7 exhibited the highest trans-3′-hydroxycotinine O-glucuronosyltransferase activity (1.1 pmol/min/mg) followed by UGT1A9 (0.3 pmol/min/mg), UGT2B15 (0.2 pmol/min/mg), and UGT2B4 (0.2 pmol/min/mg); however, trans-3′-hydroxycotinine N-glucuronidation was detected only in recombinant UGT1A4 (0.5 pmol/min/mg). As shown in Fig. 4, trans-3′-hydroxycotinine O-glucuronosyltransferase activity by recombinant UGT2B7 increased with an increase in the concentration up to 16 mM (10.5 pmol/min/mg), whereas recombinant UGT1A9 reached a plateau with a $K_m$ value of 1.6 ± 0.1 mM. The $V_{\text{max}}$ value was 0.69 ± 0.02 pmol/min/mg of protein.

Interindividual Differences of Trans-3′-Hydroxycotinine O- and N-Glucuronidations in Human Liver Microsomes and Correlation Analyses. Trans-3′-hydroxycotinine O-glucuronosyltransferase activities in microsomes from 13 human livers ranged from 2.4...
to 12.6 pmol/min/mg of protein, representing ~5-fold variability (Fig. 5A). Trans-3'-hydroxycotinine N-glucuronosyltransferase activities in microsomes from 13 human livers ranged from 6.2 to 27.1 pmol/min/mg of protein, representing ~4-fold variability (Fig. 5B). The ratio of N-glucuronide/O-glucuronide ranged from 1.0 to 4.8. Correlation analyses were performed between the trans-3'-hydroxycotinine O- or N-glucuronosyltransferase activity and imipramine (UGT1A4), propofol (UGT1A9), morphine (UGT2B7), nicotine (UGT1A4 and UGT1A9), cotinine (UGT1A4 and UGT1A9), or valproic acid (UGT2B7, UGT1A6, and UGT1A9) glucuronosyltransferase activities. Trans-3'-hydroxycotinine O-glucuronosyltransferase activities in 13 human liver microsomes were significantly ($r = 0.716, p < 0.01$) correlated with valproic acid glucuronosyltransferase activity (Table 1). Trans-3'-hydroxycotinine N-glucuronosyltransferase activity was significantly correlated with the nicotine ($r = 0.918$, $p < 0.0001$), cotinine ($r = 0.852$, $p < 0.001$), and morphine ($r = 0.798$, $p < 0.05$) glucuronosyltransferase activities.

Inhibitory Effects of Typical Substrates for UGT Isoforms on Trans-3'-Hydroxycotinine O- and N-Glucuronidation in Human

Fig. 2. Michaelis-Menten plots (A, B) or Eadie-Hofstee plots (C, D) of trans-3'-hydroxycotinine O-glucuronidation (A, C) and N-glucuronidation (B, D) in human liver microsomes. Pooled human liver microsomes were incubated with 0.25 to 16 mM trans-3'-hydroxycotinine and 2.5 mM UDPGA at 37°C for 60 min. Each data point represents the mean of duplicate determinations.

Fig. 3. Trans-3'-hydroxycotinine O-glucuronidation (A) and N-glucuronidation (B) in recombinant human UGT isoforms. Each recombinant UGT (0.5 mg/ml) was incubated with 1 mM trans-3'-hydroxycotinine and 2.5 mM UDPGA at 37°C for 60 min. Each column represents the mean of duplicate determinations.
Liver Microsomes. The effects of imipramine (UGT1A4), propofol (UGT1A9), morphine (UGT2B7), androstanediol (UGT2B15), nicotine (UGT1A4, UGT1A9), and cotinine (UGT1A4, UGT1A9) on the trans-3'-hydroxycotinine O- and N-glucuronosyltransferase activities in pooled human liver microsomes were investigated. As shown in Fig. 6, the trans-3'-hydroxycotinine O-glucuronosyltransferase activity in the pooled human liver microsomes was weakly inhibited by imipramine (IC_{50} = 55 \mu M), androstane diol (IC_{50} = 169 \mu M), and propofol (IC_{50} = 296 \mu M). The trans-3'-hydroxycotinine N-glucuronosyltransferase activity was strongly inhibited by imipramine (IC_{50} = 21 \mu M) and propofol (IC_{50} = 20 \mu M) and weakly inhibited by nicotine (IC_{50} = 178 \mu M) and androstanediol (IC_{50} = 195 \mu M).

Effects of Typical Substrates for UGT Isoforms on Trans-3'-Hydroxycotinine O-Glucuronidation in Recombinant UGT2B7 and UGT1A9. To determine the UGT isoform selectivity of inhibition by compounds, we investigated the inhibitory effects on the
trans-3'-hydroxycotinine O-glucuronidation in recombinant UGT isoforms. Trans-3'-hydroxycotinine O-glucuronosyltransferase activity in recombinant UGT2B7 was inhibited by androstanediol (IC$_{50}$ = 21 µM), propofol (IC$_{50}$ = 41 µM), and imipramine (IC$_{50}$ = 45 µM) (Fig. 7A). The inhibitory effect of morphine was weak (IC$_{50}$ = 374.2 µM). The trans-3'-hydroxycotinine O-glucuronosyltransferase activity in recombinant UGT1A9 was not affected by imipramine and morphine, whereas it was activated by propofol (2.2-fold with 500 µM) and androstanediol (500 µM) in a concentration-dependent manner (Fig. 7B).

Discussion

When the human liver microsomes were incubated with trans-3'-hydroxycotinine and UDPGA, not only O-glucuronide but also N-glucuronide were detected. The formation of N-glucuronide exceeded the formation of O-glucuronide in most human liver microsomes, being compatible with the previous report by Kuehl and Murphy (2003b). Trans-3'-hydroxycotinine N-glucuronidation in human liver microsomes was significantly correlated with nicotine and cotinine N-glucuronidations, which are catalyzed mainly by UGT1A4 (Table 1) and was inhibited by imipramine and nicotine, which are substrates of UGT1A4 (Fig. 6). Recombinant UGT1A4 exhibited substantial trans-3'-hydroxycotinine N-glucuronosyltransferase activity (Fig. 3). These results suggest that trans-3'-hydroxycotinine N-glucuronidation in human liver microsomes would be catalyzed by UGT1A4. In correlation analysis, trans-3'-hydroxycotinine N-glucuronidation in human liver microsomes was significantly correlated with morphine glucuronidation catalyzed by UGT2B7 (Table 1). However, morphine glucuronidation was also significantly correlated with nicotine ($r = 0.663, p < 0.05$) and cotinine ($r = 0.579, p < 0.05$) glucuronidations catalyzed by UGT1A4 (data not shown). Therefore, the correlation with morphine glucuronidation might be a fortuitous result with the panel of human liver microsomes used in this study. In the inhibition study, trans-3'-hydroxycotinine N-glucuronidation in human liver microsomes was inhibited by nicotine, but not by cotinine, consistent with the evidence that the $K_m$ value of cotinine N-glucuronidation (1.9 ± 0.3 mM) was higher than that of nicotine N-glucuronidation (33.1 ± 28.1 µM) (Nakajima et al., 2002b). Since trans-3'-hydroxycotinine N-glucuronidation in human liver microsomes was also inhibited by propofol (in the present study) but propofol does not inhibit UGT1A4 activity (Kuehl and Murphy, 2003a), UGT1A9 might also be involved in trans-3'-hydroxycotinine N-glucuronidation in human liver microsomes, like nicotine and cotinine N-glucuronidations (Nakajima et al., 2002b; Kuehl and Murphy, 2003a).

Trans-3'-hydroxycotinine N-glucuronidation has not been detected in smoker’s urine in vivo (Byrd et al., 1994). We also previously confirmed that trans-3'-hydroxycotinine N-glucuronide was not detected in smoker’s urine or urine samples from nonsmokers who chewed nicotine gum by LC-MS/MS analysis (data not shown). For the contradiction between in vitro and in vivo, Kuehl and Murphy (2003b) discussed the possibility that the trans-3'-hydroxycotinine N-glucuronide is unstable relative to the trans-3'-hydroxycotinine O-glucuronide and trans-3'-hydroxycotinine N-glucuronide may be cleaved prior to excretion, or alternatively, that other tissues may be the source of trans-3'-hydroxycotinine O-glucuronide formation. Thus, the reason for the contradiction between the in vitro and in vivo results has not been solved.

Trans-3'-hydroxycotinine O-glucuronosyltransferase activities were detected with the recombinant UGT2B7, UGT1A9, UGT2B15, and UGT2B4. Trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes was significantly correlated with valproic acid glucuronidation which is catalyzed by UGT2B7, UGT1A6, and UGT1A9 (Ethell et al., 2003). Trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes was inhibited by imipramine (UGT1A4), propofol (UGT1A9), and androstanediol (UGT2B15). However, it was confirmed that these three compounds inhibited trans-3'-hydroxycotinine O-glucuronosyltransferase activity catalyzed by recombinant UGT2B7 with more potent inhibitory effects than those in human liver microsomes. In addition, we found that morphine 3-glucuronosyltransferase activity catalyzed by recombinant UGT2B7 (500 µM substrate concentration) was also inhibited by 22% with 500 µM imipramine, by 37% with 500 µM propofol, and by 26% with 500 µM androstanediol (data not shown). In contrast, imipramine and androstanediol did not affect trans-3'-hydroxycotinine O-glucuronosyltransferase activity catalyzed by recombinant UGT1A9. These results suggest that a major UGT isoform involved in trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes would be UGT2B7. Although morphine glucuronidation is catalyzed by UGT2B7 (Coffman et al., 1997), the correlation with trans-3'-hydroxycotinine O-glucuronidation was not significant. It might be due to the contribution of other UGT isoforms such as UGT1A9 to the trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes. Tobacco-specific nitrosamine,
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, has been reported to be metabolized to its O-glucuronide by UGT1A9 and UGT2B7 (Ren et al., 2000) and its N-glucuronide by UGT1A4 (Wiener et al., 2004). Thus, the specificity of the UGT isoform for O- or N-glucuronidations of trans-3'-hydroxycotinine would be similar to that for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

Interestingly, trans-3'-hydroxycotinine O-glucuronidation by recombinant UGT1A9 was activated by propofol and androstanediol, although the activation was not observed with human liver microsomes. It has been reported that estradiol 3-glucuronidation in human although the activation was not observed with human liver microsomes. It has been reported that estradiol 3-glucuronidation in human liver microsomes was activated by 17α-ethynylestradiol (Williams et al., 2002). Recently, Mano et al. (2004) reported that β-estradiol and propofol activated 4-methylumbelliferone glucuronidation by recombinant UGT1A8 and UGT1A1, respectively. Thus, the heterotropic effects of some compounds on the glucuronidation have been observed. It is noteworthy that propofol inhibited 4-methylumbelliferone glucuronidation by recombinant UGT1A9 (Mano et al., 2004). Therefore, the heterotropic effects of propofol on the UGT1A9 activity would depend on the substrates.

Trans-3'-hydroxycotinine glucuronosyltransferase activities in recombinant UGTs were lower than those in human liver microsomes. Similar phenomenon has been observed in the glucuronidation of other compounds such as imipramine (Nakajima et al., 2002a), 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine (Nowell et al., 1999), and retigabine (Hiller et al., 1999). Furthermore, we previously found that recombinant UGT could not exhibit the detectable nicotine and cotinine N-glucuronidations, although human liver microsomes did show these activities (Nakajima et al., 2002b). Thus, the recombinant UGTs appear to have lower catalytic activities toward most substrates than human liver microsomes do. The fact might be partly due to the differences in the membrane circumstances in the expression system and in human liver microsomes.

The present study demonstrated that the $K_m$ value of trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes is high (ca. 10 mM), bringing up the question of the physiological significance of the reaction in vivo since blood trans-3'-hydroxycotinine concentrations are in the nanomolar range (Benowitz and Jacob, 2001). However, trans-3'-hydroxycotinine O-glucuronide is one of the major metabolites of nicotine in human urine. Generally, the $K_m$ values of the glucuronidation of various substrates are high in the millimolar range (Radominska-Pandya et al., 1999). Thus, the high $K_m$ value in vitro may suggest the possibility that in vitro experimental conditions with human liver microsomes do not reflect the activity in vivo. For drugs that are cleared predominantly through cytochrome P450-mediated metabolism, there is growing evidence that successful prediction of in vivo metabolic clearance from in vitro metabolic data can be accomplished (Iwatsubo et al., 1997). In contrast, metabolic clearance of drugs that are substantially cleared through glucuronidation appears to be less well predicted using in vitro metabolic data (Lin and Wong, 2002). It has been suggested that the latency of UGT was the main reason for the underprediction of in vivo metabolic clearance. In addition, the transport of UDPGA, removal of formed glucuronide from microsomes, the phenomena of dimerization of UGT, and glucuronide hydrolysis have been also suggested to contribute to the underprediction (Lin and Wong, 2002).

In conclusion, we thoroughly characterized the trans-3'-hydroxycotinine O- and N-glucuronidations in human liver microsomes. This is the first study to show that trans-3'-hydroxycotinine O-glucuronidation in human liver microsomes is catalyzed mainly by UGT2B7 and, to a minor extent, by UGT1A9.

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References


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