CHARACTERIZATION OF NICOTINE AND COTININE N-GLUCURONIDATIONS IN HUMAN LIVER MICROSOMES

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ABSTRACT:

The nicotine and cotinine N-glucuronidations in human liver microsomes were determined. The Eadie-Hofstee plots of nicotine N-glucuronidation in human liver microsomes were clearly biphasic, indicating the involvement of multiple enzymes. The apparent K_m and V_max values were 33.1 ± 28.1 μM and 60.0 ± 21.0 pmol/min/mg and 284.7 ± 122.0 μM and 124.0 ± 44.0 pmol/min/mg for the high- and low-affinity components, respectively, in human liver microsomes (n = 4). However, the Eadie-Hofstee plots of cotinine N-glucuronidation in human liver microsomes were monophasic (apparent K_m = 1.9 ± 0.3 mM, V_max = 655.6 ± 312.3 pmol/min/mg). The nicotine and cotinine N-glucuronidations in the recombinant human UDP-glucuronosyltransferases (UGTs) (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15) expressed in baculovirus-infected insect cells or human B-lymphoblastoid cells that are commercially available were determined. However, no recombinant UGT isoforms showed detectable nicotine and cotinine N-glucuronides (the concentrations of nicotine and cotinine were 0.5 and 2 mM, respectively). Nicotine and cotinine N-glucuronidations in pooled human liver microsomes were competitively inhibited by bilirubin as a substrate for UGT1A1 (K_i = 3.9 and 3.3 μM), imipramine as a substrate for UGT1A4 (K_i = 6.1 and 2.7 μM), and propofol as a substrate for UGT1A9 (K_i = 6.0 and 12.0 μM). The nicotine N-glucuronidation (50 μM nicotine) in 14 human liver microsomes was significantly (r = 0.950, P < 0.0001) correlated with the cotinine N-glucuronidation (0.2 mM cotinine), indicating that the same isoform(s) is involved in both glucuronidations. Furthermore, weak correlations between imipramine N-glucuronidation and nicotine N-glucuronidation (r = 0.425) or cotinine N-glucuronidation (r = 0.517) were observed. In conclusion, the involvement of UGT1A1 and UGT1A9 as well as UGT1A4 in nicotine and cotinine N-glucuronidations in human liver microsomes was suggested, although the contributions of each UGT isoform could not be determined conclusively.

Nicotine is mainly metabolized to cotinine by CYP2A6 in human livers (Nakajima et al., 1996a), and cotinine is further metabolized to trans-3′-hydroxycotinine by CYP2A6 (Nakajima et al., 1996b). Nicotine and cotinine have been used to assess nicotine intake in smokers and nonsmokers (Kyerematen et al., 1990; Benowitz et al., 1994). Although 81 to 98% of the nicotine absorbed from tobacco smoking is recovered in the urine as nicotine and its metabolites (Kyerematen et al., 1990), urinary nicotine and cotinine account for only 10 to 40% of the nicotine absorbed (Neurath et al., 1988). Glucuronidation is an important pathway of nicotine metabolism in humans. The average percentages of nicotine N-glucuronide and cotinine N-glucuronide excreted in smoker’s urine were approximately 3 to 4% and 9 to 17% of nicotine absorbed, respectively (Curvall et al., 1991; Byrd et al., 1992; Benowitz et al., 1994).

In humans, there are large interindividual differences in nicotine metabolism (Cholerton et al., 1994; Benowitz et al., 1995). Recently, we reported that the genetic polymorphism of CYP2A6 affects the nicotine formation from nicotine (Kwon et al., 2001; Nakajima et al., 2001). Similarly, the metabolic pathway of glucuronidation would be one of the causal factors for the interindividual differences in nicotine metabolism. Indeed, considerable interindividual variability in the percentages of the conjugates of nicotine (3.8–56.0%) and cotinine (0–60.3%) in urine has been reported (Benowitz et al., 1994). It has also been reported that nicotine and cotinine N-glucuronidations seemed to be polymorphic in African-Americans, although they were unimodal in Caucasians (Benowitz et al., 1999).

Glucuronidation is catalyzed by UDP-glucuronosyltransferases (UGTs1) (Miners and Mackenzie, 1991). It is well known that there are many isoforms of mammalian UGT enzymes (Tukey and Strassburg, 2000). To date, two UGT families have been identified in humans: UGT1 and UGT2. The UGT1 and UGT2 genes seem to be structurally different in that UGT1 proteins result from alternate splicing of different first exons with five shared exons encoded by the UGT1 gene complex, whereas UGT2 proteins seem 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). In general, it is known that only human UGT1A3 and UGT1A4 catalyze the N-glucuronidation of tertiary amines, such as imipramine,

1 Abbreviations used are: UGT, UDP-glucuronosyltransferase; HPLC, high-performance liquid chromatography; PAH, polycyclic aromatic hydrocarbon.
amitriptyline, and chlorpromazine, to form quaternary ammonium glucuronides (Green et al., 1995; Green and Tephly, 1998). Quaternary ammonium glucuronides are formed from nicotine and cotinine. However, the UGT isoforms that catalyze the nicotine and cotinine glucuronidations have not been determined. Therefore, the purpose of the present study is to identify the human UGT isoform involved in nicotine and cotinine N-glucuronidations. Previously, we established a highly sensitive HPLC-UV method for directly determining nicotine N-glucuronide and cotinine N-glucuronide (Nakajima et al., 2002a). We applied this method for determining the N-glucuronosyltransferase activities of nicotine and cotinine in human liver microsomes.

Materials and Methods

Materials. (S)-Nicotine, (S)-cotinine, UDP-glucuronic acid, alamethicin, and estradiol were purchased from Sigma-Aldrich (St. Louis, MO). Nicotine N-β-glucuronide and cotinine N-β-glucuronide were purchased from Toronto Research Chemicals (Toronto, ON, Canada). 4-Nitrophenol, imipramine hydrochloride, and bilirubin were from Wako Pure Chemicals (Osaka, Japan). Morphine hydrochloride was purchased from Tekeda Chemical Industries (Osaka, Japan). Propofol was kindly supplied by AstraZeneca (London, UK). 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 (Woburn, MA). The glucuronosyltransferase activities of estradiol, trifluoperazine, and propofol as typical activities for UGT1A1, UGT1A4, and UGT1A19, respectively, in these human liver microsomes except for H161, H006, H030, and H070 were provided by the manufacturer. Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15 expressed in baculovirus-infected insect cells (Supersomes) were provided from Gentest. Recombinant UGT1A1, UGT1A4, UGT1A6, and UGT1A9 expressed in human B-lymphoblastoid cells were also from Gentest. Recombinant UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A10, and UGT2B7 expressed in baculovirus-infected insect cells (Baculosomes) were from PanVera (Madison, WI). All other chemicals and solvents were of the highest grade commercially available.

Nicotine and Cotinine N-Glucuronidation Assays. These assays were previously established in our laboratory (Nakajima et al., 2002a). Briefly, a typical incubation mixture (200 μl of total volume) contained 20 mM Tris-HCl buffer, pH 7.4, 5 mM MgCl₂, 2.5 mM UDP-glucuronic acid, 25 μg/ml alamethicin, 0.25% (v/v) human liver microsomes, and 50 μM nicotine or 0.2 mM cotinine. For recombinant UGTs, 0.5 mg/ml microsomes and 0.5 mM nicotine or 2 mM cotinine were used. The reactions were initiated by the addition of UDP-glucuronic acid and were then incubated at 37 °C for 30 min. The reactions were terminated by boiling for 10 min. After removal of the protein by centrifugation at 10,000 rpm for 5 min, a 2-μl solution containing phosphoric acid and heptane sulfonate sodium for nicotine N-glucuronide or octane sulfonate sodium for cotinine N-glucuronide was added to make the concentrations of these chemicals the same as those in the mobile phases. A 20-μl portion of the sample was subjected to HPLC. Chromatography was performed using an L-7100 pump (Hitachi, Tokyo, Japan), an L-7400 UV detector (Hitachi), an L-7200 autosampler (Hitachi), an L-7500 integrator (Hitachi), and an 865-CP column oven (Jasco, Tokyo, Japan) with a Capcell Pak C₁₈, UG120 (4.6 × 250 mm; 4 μm) column (Shiseido, Tokyo, Japan). The flow rate was 1.0 ml/min and the column temperature was 35 °C. The eluent was monitored at 260 nm with a noise-base clean Uni-3 (Union, Gunma, Japan). The Uni-3 can reduce the noise by integration of the output and further amplification with an internal amplifier, resulting in a maximum 15-fold amplification of the signal. The mobile phases were 3% CH₃OH, 2 mM NaH₂PO₄, 0.2% phosphoric acid, and 4 mM heptane sulfonate sodium for the determination of the nicotine N-glucuronide, and 1% CH₃OH, 2 mM NaH₂PO₄, 0.1% phosphoric acid, and 5 mM octane sulfonate sodium for the determination of the cotinine N-glucuronide. The quantification of nicotine N-glucuronide or cotinine N-glucuronide was performed by comparing the HPLC peak heights to those of authentic standards.

Kinetic Analyses of Nicotine and Cotinine N-Glucuronidations in Human Liver Microsomes. Kinetic parameters were determined with the assays described above. The concentrations of nicotine and cotinine were 25 μM to 1 mM and 50 μM to 10 mM, respectively. Kinetic parameters (apparent Kᵣ and Vₘₐₓ) were estimated by analyzing the Eadie-Hofstee plots.

Inhibition Analysis of Nicotine and Cotinine N-Glucuronidations in Human Liver Microsomes. Six compounds were tested for their inhibitory effects on the nicotine and cotinine N-glucuronidations in pooled human liver microsomes. Bilirubin is a typical substrate for UGT1A1 (Bosma et al., 1994; Senafi et al., 1994). Estradiol is a substrate for UGT1A1 (and UGT1A9 as a minor enzyme) (Bosma et al., 1994; Senafi et al., Hanioka et al., 2001b). 4-Nitrophenol is a substrate for UGT1A6 and UGT1A9 (Hanioka et al., 2001a,b). Imipramine is a substrate for UGT1A4 (and UGT1A3 as a minor enzyme) (Green et al., 1995, 1998; Green and Tephly, 1998). Propofol is a specific substrate for UGT1A9 (McGurk et al., 1998; Hanioka et al., 2001b). Morphine is a typical substrate for UGT2B7 (Coffman et al., 1997). Furthermore, the inhibitory effects of cotinine on nicotine N-glucuronidation and those of nicotine on cotinine N-glucuronidation in human liver microsomes were also determined. For the determination of the IC₅₀ values, the concentrations of nicotine and cotinine were set at 50 μM and 0.2 mM, respectively. For determination of the Kᵢ values, the concentrations of nicotine and cotinine were 50 to 500 μM and 0.3 to 10 mM, respectively. The Lineweaver-Burk plot and Dixon plot were adopted to determine the Kᵢ value and the inhibitory type. Bilirubin and 4-nitrophenol were dissolved in dimethyl sulfoxide and ethanol, respectively. Estradiol and propofol 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).

Imipramine Glucuronidation Assay. The assay was previously established in our laboratory (Nakajima et al., 2002c). Briefly, a typical incubation mixture (200 μl of 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, and 0.5 mM imipramine. The reactions were initiated by the addition of UDP-glucuronic acid and were then incubated at 37 °C for 60 min. The reactions were 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. The instruments of HPLC were described above. Chromatographic separations were performed on a Mighyssill RP-18 (4.6 × 150 mm; 5 μm) column (Kanto Chemical, 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 4.0. The retention times of imipramine N-glucuronide and imipramine were 6.2 and 15.4 min, respectively. The peak of imipramine N-glucuronide was confirmed by liquid chromatography-mass/mass spectrometry analysis (Nakajima et al., 2002c).

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.

Correlation Analyses. Correlations between Nicotine N-glucuronidation and cotinine N-glucuronidations, 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

Kinetic Parameters of Nicotine and Cotinine N-Glucuronidations in Human Liver Microsomes. Kinetic analyses were performed using pooled human liver microsomes (H161) and representative liver microsomes from four humans (H003, H030, H042, and H112). The Eadie-Hofstee plots for nicotine N-glucuronidation in all human liver microsomes examined in this study were clearly biphasic. A representative plot is shown in Fig. 1A. The apparent Kᵣ values were 33.1 ± 28.1 and 284.7 ± 122.0 μM for the high- and low-affinity components, respectively, in the liver microsomes from four humans (Table 1). The apparent Vₘₐₓ values were 60.0 ± 21.0 and 124.0 ± 44.0 pmol/min/mg for the high- and low-affinity compo-
materials and methods were used to determine their nicotine and cotinine gluturonosyltransferase activities. No recombinant UGT isoforms expressed in human B-lymphoblastoid cells or baculovirus-infected insect cells from four humans. Similar values were also obtained with the pooled human liver microsomes.

Nicotine and Cotinine N-Glucuronidations in Recombinant Human UGT Isoforms. All recombinant UGT isoforms expressed in human B-lymphoblastoid cells or baculovirus-infected insect cells that are commercially available were used to determine their nicotine and cotinine N-glucuronosyltransferase activities. No recombinant UGT isoform exhibited detectable nicotine N-glucuronidation nor cotinine N-glucuronide formations.

Inhibitory Effects of Typical Substrates for UGT Isoforms on Nicotine and Cotinine N-Glucuronidations in Human Liver Microsomes. The effects of bilirubin (UGT1A1), estradiol (UGT1A1 and UGT1A9), imipramine (UGT1A3 and UGT1A4), 4-nitrophenol (UGT1A6 and UGT1A9), propofol (UGT1A9), and morphine (UGT2B7) on nicotine and cotinine N-glucuronidations in pooled human liver microsomes were determined. Furthermore, the inhibitory effects of cotinine on nicotine N-glucuronidation and those of nicotine on cotinine N-glucuronidation in human liver microsomes were also determined. Nicotine N-glucuronidation in the pooled human liver microsomes was inhibited by bilirubin (IC$_{50}$ = 1.9 μM), propofol (IC$_{50}$ = 13.4 μM), estradiol (IC$_{50}$ = 45.0 μM), and imipramine (IC$_{50}$ = 48.3 μM). The inhibitory effects of 4-nitrophenol (IC$_{50}$ = 84.0 μM), morphine (IC$_{50}$ > 1 mM), and cotinine (IC$_{50}$ > 1 mM) were not prominent. Cotinine N-glucuronidation in the pooled human liver microsomes was inhibited by bilirubin (IC$_{50}$ = 1.6 μM), imipramine (IC$_{50}$ = 2.4 μM), propofol (IC$_{50}$ = 14.2 μM), estradiol (IC$_{50}$ = 52.7 μM), and nicotine (IC$_{50}$ = 74.5 μM). The inhibitory effects of 4-nitrophenol could not be determined because of the interference from the peak of cotinine N-glucuronide. The effects of morphine were not prominent (IC$_{50}$ > 1 mM).

The $K_i$ value and inhibitory type of bilirubin, imipramine, and propofol for nicotine and cotinine in human liver microsomes were determined. As shown in Fig. 2A, C, and E, nicotine N-glucuronida-

![Typical Eadie-Hofstee plots of nicotine N-glucuronidation (A) and cotinine N-glucuronidation (B) in human liver microsomes (H030). The concentrations of nicotine and cotinine ranged from 25 μM to 1 mM, respectively. The formations of nicotine N-glucuronide and cotinine N-glucuronide were determined as described under Materials and Methods. Each data point represents the mean of duplicate determinations.](image-url)
tion in pooled human liver microsomes was competitively inhibited by bilirubin \( (K_i = 3.9 \, \mu M) \), imipramine \( (K_i = 6.1 \, \mu M) \), and propofol \( (K_i = 6.0 \, \mu M) \). Cotinine \( \text{N}-\text{glucuronidation} \) in pooled human liver microsomes was also competitively inhibited by bilirubin \( (K_i = 3.3 \, \mu M) \), imipramine \( (K_i = 2.7 \, \mu M) \), and propofol \( (K_i = 12.0 \, \mu M) \) (Fig. 2, B, D, and F).

**Interindividual Variability of Nicotine and Cotinine \( \text{N}-\text{Glucuronidations} \) in Human Liver Microsomes.** Nicotine \( \text{N}-\text{glucuronidation} \) activities in microsomes from 14 human livers ranged from 3.3 to 71.1 pmol/min/mg, representing \( 22 \)-fold variability (Fig. 3A). Cotinine \( \text{N}-\text{glucuronidation} \) activities in microsomes from 14 human livers ranged from 3.5 to 310.1 pmol/min/mg, representing \( 89 \)-fold variability (Fig. 3B).

**Correlation Analyses.** As shown in Table 3, the nicotine \( \text{N}-\text{glucuronidation} \) activities in liver microsomes from 14 humans were significantly correlated with the cotinine \( \text{N}-\text{glucuronidation} \) activities \( (r = 0.950, P < 0.001) \). Weak correlations between imipramine \( \text{N}-\text{glucuronidation} \) and nicotine \( \text{N}-\text{glucuronidation} \) \( (r = 0.425) \) or cotinine \( \text{N}-\text{glucuronidation} \) \( (r = 0.517) \) were observed, although these were not statistically significant. The imipramine \( \text{N}-\text{glucuronidation} \) activity measured in the present study was significantly correlated with the trifluoperazine glucuronidation activities provided by the manufacturer \( (r = 0.816, P < 0.005) \). The nicotine and cotinine \( \text{N}-\text{glucuronidation} \) activities did not correlate with the estradiol (UGT1A1), trifluoperazine (UGT1A4), and propofol (UGT1A9) glucuronidations (Table 3).
Discussion

The glucuronidations of nicotine and cotinine are important metabolic pathways of nicotine in humans. It has been reported that the percentages of nicotine N-glucuronide and cotinine N-glucuronide excreted in urine were quite variable among smokers (Benowitz et al., 1994; Byrd et al., 2000). This considerable interindividual variability in nicotine and cotinine N-glucuronidations would be one of the factors in the large interindividual differences in nicotine metabolism. In the present study, the nicotine and cotinine N-glucuronidations in human liver microsomes were thoroughly characterized.

In our preliminary study, the nicotine and cotinine N-glucuronidations in human liver microsomes were determined at pH 7.4, 8.0, and 8.4 in 20 mM Tris-HCl buffer. However, no effects of the pH were observed, although Ghosheh et al. (2001) recently reported that nicotine N-glucuronidation was dramatically increased 6-fold over a pH range of 7.4 to 8.4. In contrast, we experienced that high concentrations of salt (100 mM Tris-HCl and 10 mM MgCl₂) decreased the formations of nicotine N-glucuronide or cotinine N-glucuronide by about 50%. Therefore, the formations of nicotine N-glucuronide or cotinine N-glucuronide in human liver microsomes were determined at pH 7.4 with 20 mM Tris-HCl and 5 mM MgCl₂ in the present study. Very recently, it has been reported that the kinetics of nicotine N-glucuronidation in human liver microsomes was monophasic with a Kₘ value of 0.11 ± 0.017 mM (Ghosheh et al., 2001). However, in our study, the kinetics was clearly biphasic, indicating the involvement of multiple enzymes. Differences between the assay conditions of their method (50 mM Tris-HCl, pH 8.4, 5 mM MgCl₂, 1 g/ml alamethicin, and 2 mM UDP-glucuronic acid in their assay) and those of the present study might affect the kinetic profile of nicotine N-glucuronidation.

It has been reported that the extent of conjugation of nicotine and cotinine excreted in urine was highly correlated in an in vivo study (Benowitz et al., 1994). The present study is the first to show the highly significant correlation between nicotine N-glucuronidation and cotinine N-glucuronidation in human liver microsomes in an in vitro study. These results suggest that the same enzymes are involved in the glucuronidations of nicotine and cotinine. It has been reported that the N-glucuronidations of tertiary amines are catalyzed by UGT1A3 and UGT1A4 (Green et al., 1995; Green and Tephly, 1998). However, it is somewhat doubtful whether UGT1A3 makes a significant contribution to the overall metabolism of tertiary amines because of the higher apparent Kₘ values and lower expression in human liver compared with UGT1A4 (Green and Tephly, 1998). Until now, it has been uncertain whether UGTs other than UGT1A3 and UGT1A4 catalyze the N-glucuronidation of tertiary amines. However, in the present study, the nicotine and cotinine N-glucuronidations in human liver microsomes were competitively inhibited by bilirubin, imipramine, and propofol, indicating the involvement of UGT1A1, UGT1A9, and other UGTs.
UGT1A4, and UGT1A9. This is the first report that UGT1A1 and UGT1A9 possibly catalyze the \(N\)-glucuronidation of tertiary amines. The nicotine and cotinine \(N\)-glucuronidations were weakly correlated with the imipramine \(N\)-glucuronidation catalyzed by mainly UGT1A4 rather than with glucuronidations of estradiol and propofol. Therefore, it was considered that some contributions of UGT1A1 and UGT1A9 to the nicotine and cotinine \(N\)-glucuronidations might decrease the correlation coefficients with imipramine \(N\)-glucuronidation. The trifluoperazine glucuronidation is also considered to be catalyzed by UGT1A4 (Green and Tephly, 1996) and is significantly correlated with imipramine \(N\)-glucuronidation \((r = 0.816, P < 0.005)\). The reason that there were no correlations between the nicotine or cotinine \(N\)-glucuronidations and the trifluoperazine glucuronidation is unknown.

To identify the UGT isoform(s) involved in nicotine and cotinine \(N\)-glucuronidations, the activities of recombinant UGTs expressed in human B-lymphoblastoid cells or baculovirus-infected insect cells that were commercially available were determined. However, no recombinant UGT isoform showed nicotine nor cotinine \(N\)-glucuronidation. We confirmed that these recombinant UGTs were active for other substrates such as imipramine and troglitazone (Nakajima et al., 2002c; Watanabe et al., 2002). Therefore, it was indicated that the turnovers of nicotine and cotinine \(N\)-glucuronidations might be lower than those of other substrates in these recombinant UGTs. Unfortunately, the contributions of each UGT isoform to nicotine and cotinine \(N\)-glucuronidation in human liver microsomes could not be directly estimated. Furthermore, we recently reported that the all recombinant UGTs did not exhibit the glucuronidation of 5-(4'-hydroxyphenyl)-5-phenylhydantoin, a major metabolite of phenytoin (Nakajima et al., 2002). In addition, we clarified that the morphine 3-glucuronosyltransferase activity in recombinant UGT2B7 was 25-fold lower than that in the pooled in human liver microsomes (data not shown). The differences in these glucuronosyltransferase activities between the recombinant UGTs and human liver microsomes might be partly due to the differences in the membrane circumstances in the expression system and in human liver microsomes. Exactly, it has been reported that the nature of the phospholipid environment influences the rate-limiting step of glucuronidation (Magdalou et al., 1982).

It has been reported that monoglucuronidation of phenols may be catalyzed by a dimeric form of UGT, whereas diglucuronidation is catalyzed by a tetramer (Gschaidmeier and Bock, 1994). Homooligomer formation of rat UGT2B1 (Meech and Mackenzie, 1997) and heterooligomer formation of rat UGT2B1 and UGT1A family (Ikushiro et al., 1997) have been reported. Recently, Ishii et al. (2001) reported that heterooligomer formation of guinea pig UGT2B21 and UGT2B22 leads to altered substrate specificity. In recombinant UGTs used in most studies, including the present study, only homo-oligomers would be formed. In the future studies, it is necessary to elucidate whether the heterooligomers with many different combinations can exhibit the catalytic activities of nicotine or cotinine glucuronidations by double-transfection of different UGT cDNAs.

Large interindividual variability in nicotine \(N\)-glucuronidation (~22 fold) and cotinine \(N\)-glucuronidation (~89 fold) in human liver microsomes was observed in the present study. Therefore, it is suggested that the large interindividual differences in the percentages of glucuronide conjugates excreted in urine were due to the interindividual differences in the formations of these metabolites, i.e., the catalytic properties of the UGT isoforms, rather than the process of excretion. It has been reported that nicotine and cotinine \(N\)-glucuronidations seemed to be polymorphic in African-Americans, although these were unimodal in Caucasians (Benowitz et al., 1999). There are genetic polymorphisms in certain UGT isoforms, i.e., UGT1A1, UGT1A6, UGT2B4, UGT2B7, and UGT2B15 (Tukey and Strassburg, 2001). A mutation in UGT1A4 has also been found, although its clinical significance is unknown (Burchell et al., 1994). Therefore, the genetic polymorphisms in UGT1 isoforms might be a cause of the interindividual differences in nicotine and cotinine \(N\)-glucuronidation in humans. Furthermore, it has been reported that UGT1A6 and UGT1A9 are inducible by polycyclic aromatic hydrocarbons (PAHs) (Bock et al., 1999). Cigarette smoke contains abundant PAHs such as benzo[a]pyrene, benzo[a]anthanthrene. Thus, the inducibility of UGTs by PAHs in cigarette smoke might also be one of the causes of interindividual differences in nicotine and cotinine \(N\)-glucuronidations.

In conclusion, nicotine and cotinine \(N\)-glucuronidations in human liver microsomes were characterized thoroughly. The kinetics of nicotine \(N\)-glucuronidation in human liver microsomes was clearly biphasic, whereas that of cotinine \(N\)-glucuronidation was monophasic. Based on the highly significant correlation between the nicotine \(N\)-glucuronidation and cotinine \(N\)-glucuronidation in human liver microsomes, the same UGT isoform(s) might be involved in these glucuronidations. In addition to the contribution of UGT1A4 to the nicotine and cotinine \(N\)-glucuronidations in human liver microsomes, the involvement of UGT1A1 and UGT1A9 was also implicated.

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References


Meech R and Mackenzie PI (1997) UDP-glucuronosyltrasnferase, the role of the amino terminus
Magdalou J, Hochman Y, and Zakim D (1982) Factors modulating the catalytic specificity of a
Nakajima M, Kwon J-T, Tanaka E, and Yokoi T (2002a) High-performance liquid chromato-
Nakajima M, Kwon J-T, Tanaka N, Zenta T, Yamamoto Y, Yamamoto H, Yamazaki H,
Ishii Y, Miyoshi A, Watanabe R, Tsuruda K, Tsuda M, Yamaguchi-Nagamatsu Y, Yoshide K,
Hawes EM (1998) Nicotine metabolism and eight metabolites that are longer lived than cotinine.
Koreans.