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Review

Interindividual Variability in Nicotine Metabolism: C-Oxidation and Glucuronidation

Miki NAKAJIMA and Tsuyoshi YOKOI

Drug Metabolism and Toxicology, Division of Pharmaceutical Sciences, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

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Summary: Nicotine has roles in the addiction to smoking, replacement therapy for smoking cessation, as a potential medication for several diseases such as Parkinson's disease, Alzheimer's disease, and ulcerative colitis. The absorbed nicotine is rapidly and extensively metabolized and eliminated to urine. A major pathway of nicotine metabolism is *C*-oxidation to cotinine, which is catalyzed by CYP2A6 in human livers. Cotinine is subsequently metabolized to *trans*-3′-hydroxycotinine by CYP2A6. Nicotine and cotinine are glucuronidated to *N*-glucuronides mainly by UGT1A4 and partly by UGT1A9. *Trans*-3′-hydroxycotinine is glucuronidated to *O*-glucuronide mainly by UGT2B7 and partly by UGT1A9. Approximately 90% of the total nicotine uptake is eliminated as these metabolites and nicotine itself. The nicotine metabolism is an important determinant of the clearance of nicotine. Recently, advances in the understanding of the interindividual variability in nicotine metabolism have been made. There are substantial data suggesting that the large interindividual differences in cotinine formation are associated with genetic polymorphisms of the *CYP2A6* gene. Interethnic differences have also been observed in the cotinine formation and the allele frequencies of the *CYP2A6* alleles. Since the genetic polymorphisms of the *CYP2A6* gene have a major impact on nicotine clearance, its relationships with smoking behavior or the risk of lung cancer have been suggested. The metabolic pathways of the glucuronidation of nicotine, cotinine, and *trans*-3′-hydroxycotinine in humans would be one of the causal factors for the interindividual differences in nicotine metabolism. This review mainly summarizes recent results from our studies.

Key words: CYP2A6; UDP-glucuronosyltransferase; genetic polymorphism; interindividual difference; ethnic difference

Introduction

Over a billion people worldwide smoke tobacco. Smoking exerts complex central and peripheral nervous system, behavioral, cardiovascular, and endocrine effects in humans.[1,2] Smoking is associated with a higher incidence of various types of cancers, respiratory and cardiovascular disease, gastrointestinal disorders as well as many other medical complications.[3] The addiction liability and pharmacological effects of smoking are due to nicotine. Pulmonary absorption of nicotine is extremely rapid, occurring at a rate similar to that after intravenous administration.[4] The absorbed nicotine is rapidly and extensively metabolized and eliminated to urine.[2]

In humans, a major pathway of nicotine metabolism is *C*-oxidation to cotinine (**Fig. 1**), which is catalyzed by hepatic cytochrome P4502A6 (CYP2A6).[5] Cotinine is subsequently hydroxylated to *trans*-3′-hydroxycotinine by CYP2A6.[6] Nicotine, cotinine, and *trans*-3′-hydroxycotinine are glucuronidated to nicotine *N*-glucuronide, cotinine *N*-glucuronide, and *trans*-3′-hydroxycotinine *O*-glucuronide, respectively. Approximately 85–95% of the total nicotine uptake is eliminated as nicotine, cotinine, *trans*-3′-hydroxycotinine, and their glucuronides in urine.[7,8] Other minor metabolites are nicotine 1′-*N*-oxide, cotinine 1′-*N*-oxide, nornicotine, and norcotinine. Although the nicotine 1′-*N*-oxide formation from nicotine has been reported to be catalyzed by flavin-containing monooxygenase 3 (FMO3),[9] the enzyme(s) responsible for cotinine 1′-*N*-oxide formation from cotinine is unknown. Norcotinine formation

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To whom all correspondence should be sent: Miki NAKAJIMA, Ph.D., *Drug Metabolism and Toxicology, Division of Pharmaceutical Sciences, Graduate School of Medical Science, Kanazawa University, Kakuma-machi, Kanazawa 920–1192, Japan. Tel. & Fax. +81-76-234-4407, E-mail: nmiki@kenroku.kanazawa-u.ac.jp*

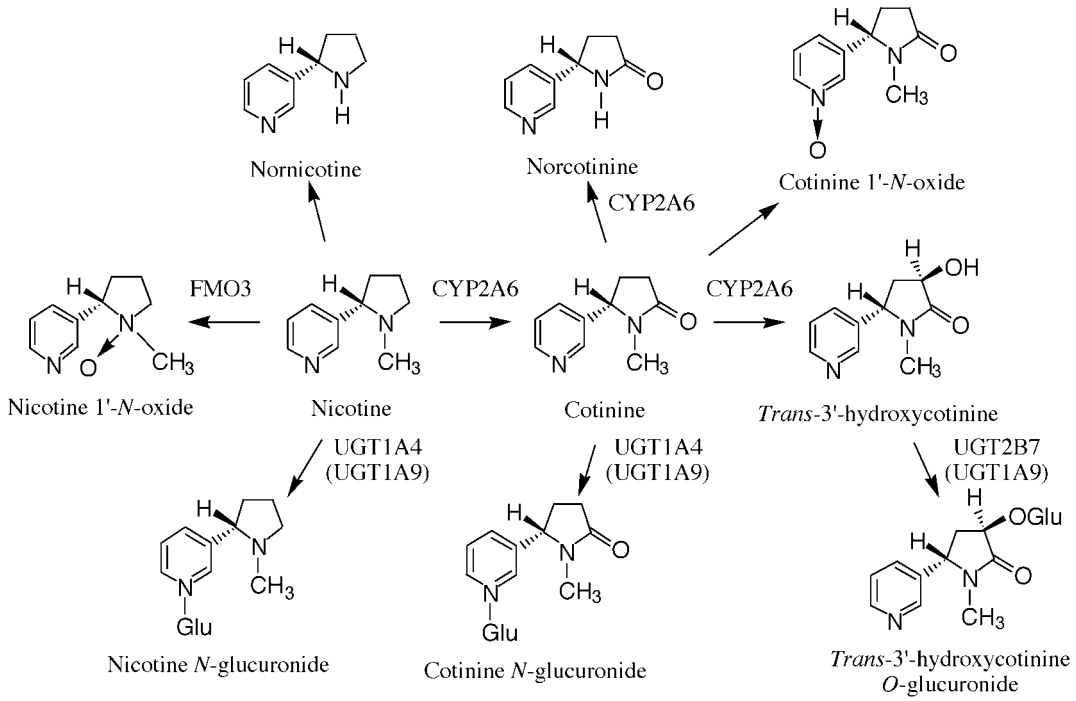

Fig. 1. Metabolic pathways of nicotine in humans.

from cotinine is catalyzed by CYP2A6,[10] but the enzyme(s) responsible for nornicotine formation from nicotine is unknown.

Hepatic metabolism is the primary route of elimination of nicotine. Variability in the nicotine metabolism could be an important determinant of the clearance of nicotine. This review focuses on the interindividual differences in the major metabolic pathways, *C*-oxidation and glucuronidation, of nicotine in humans.

Interindividual Differences in Cotinine Formation and Genetic Polymorphisms of *CYP2A6* Gene

Typically, 70–80% of the absorbed nicotine is metabolized to cotinine. Large interindividual variability in the cotinine formation has been demonstrated.[11–15] The half-lives of nicotine and cotinine after smoking are approximately 2 hr and 20 hr, respectively.[16] Because of the longer half-life of cotinine, it is generally used as a biomarker of smoking. Thus, the evaluation of the metabolic potency of nicotine to cotinine in ordinary smokers would not be easy, since the cotinine levels are reflected by the extent of smoking (the number of cigarettes smoked, nicotine content per one cigarette, the depth of inhalation, and the force of drawing). We developed a simple and noninvasive method for phenotyping of nicotine metabolism to cotinine in non-smokers.[17] If smokers could abstain from smoking for about 2 weeks, this phenotyping method would be applicable. In the phenotyping method, after the subjects chewed one piece of nicotine gum, the concentrations of nicotine and cotinine in the plasma 2 hr after the chewing were determined. Consequently, large interindividual differences were observed in the cotinine/nicotine ratios calculated as the metabolic index. In Japanese (n = 92) and Korean (n = 209) populations, the cotinine/nicotine ratios ranged from 0 to 14.7 and 0 to 143.9, respectively.[18,19] Similar large interindividual differences have been also observed in European-American and African-American populations with this phenotyping method. The results of our comprehensive analyses for interindividual and interethnic differences in cotinine formation will soon be reported.

Cotinine formation from nicotine is catalyzed by CYP2A6.[5] There is genetic polymorphism in the human *CYP2A6* gene (http://www.imm.ki.se/CYPalleles/cyp2a6.htm). Several mutated alleles have been reported to decrease or delete the enzymatic activity (**Table 1**). *CYP2A6*4* allele, in which the entire *CYP2A6* gene is deleted, completely lacks the enzymatic activity.[20–22] Alleles possessing a single nucleotide polymorphism (SNP), such as *CYP2A6*2* (L160H),[23] *CYP2A6*5* (G479V),[24] *CYP2A6*6* (R128Q),[14] *CYP2A6*7* (I471T),[25] *CYP2A6*9* (T-48G in TATA box),[26] *CYP2A6*10*,[15,27] and *CYP2A6*11* (S224P),[28] have been reported to lead to decreased enzymatic activity *in vitro* and/or *in vivo*. In our studies, the relationship between the interindividual differences in nicotine metabolism to cotinine and genetic polymorphisms of the *CYP2A6* gene in Japanese and Korean subjects was

Table 1. Characteristics of human *CYP2A6* allele

Allele	*Nucleotide changes*		*Effect*	*Enzyme activity*	
	cDNA	Gene		*In vivo*	*In vitro*
*CYP2A6*1A*	None	None		Normal	Normal
*CYP2A6*1B*	gene conversion in the 3′-flanking region	−1013 A>G: gene conversion in the 3′-flanking region			
*CYP2A6*1C*	gene conversion in the 3′-flanking region	−395 G>A: gene conversion in the 3′-flanking region			
*CYP2A6*1D*		−1013 A>G			
*CYP2A6*1E*	gene conversion in the 3′-flanking region				
*CYP2A6*1F*	1224 C>T	5717 C>T			
*CYP2A6*1G*	1224 C>T	5717 C>T; 5825 A>G			
*CYP2A6*1H*		−745 A>G			
*CYP2A6*1J*		−1013 A>G; −745 A>G			
*CYP2A6*1X2*			gene duplication		
*CYP2A6*2*	51 G>A; 479 T>A	51 G>A; 1799 T>A	L160H	None	None
*CYP2A6*3*	*CYP2A6*/*CYP2A7* hybrid				
*CYP2A6*4*	*CYP2A6* deleted		CYP2A6 deleted	None	
*CYP2A6*5*	1436 G>T; gene conversion in the 3′-flanking region	6582 G>T; gene conversion in the 3′-flanking region	G479V	None	None
*CYP2A6*6*	383 G>A	1703 G>A	R128Q		Decreased
*CYP2A6*7*	1412 T>C; gene conversion in the 3′-flanking region	6558 G>C; gene conversion in the 3′-flanking region	I471T	Decreased	Decreased
*CYP2A6*8*	1454 G>T; gene conversion in the 3′-flanking region	6600 G>T gene conversion in the 3′-flanking region	R485L	Normal	
*CYP2A6*9*		−1013 A>G; −48 T>G	TATA box	Decreased	Decreased
*CYP2A6*10*	1412 T>C; 1454 G>T; gene conversion in the 3′-flanking region	6558 T>C; 6600 G>T; gene conversion in the 3′-flanking region	I471T; R485L	Decreased	
*CYP2A6*11*	670 T>C	3391 T>C	S224P	Decreased	Decreased
*CYP2A6*12*	exons 1-2 of *CYP2A7* origin; exons 3-9 of *CYP2A6* origin		10 amino acid substitutions	Decreased	Decreased
*CYP2A6*13*	13 G>A	−48 T>G; 13 G>A	G5R		
*CYP2A6*14*	51 G>A; 86 G>A	51 G>A; 86 G>A	S29N		
*CYP2A6*15*	580 A>G	−48 T>G; 2134 A>G	K194E		
*CYP2A6*16*	607 C>A	2161 C>A	R203S		
*CYP2A6*17*	459 G>A; 1093 G>A; 1224 C>T	209>T; 1779 G>A; 4489 C>T; 5065 G>A; 5163 G>A; 5717 C>T; 5825 A>G	V365M	Decreased	Decreased
*CYP2A6*18*	1175 A>T	5668 A>T	Y392F		Decreased
*CYP2A6*19*	1175 A>T; 1412 T>C; gene conversion in the 3′-flanking region	5668 A>T; 6354 T>C; 6558 T>C; gene conversion in the 3′-flanking region	Y392F; I471T		Decreased
*CYP2A6*20*	51 G>A; 587-588delAA; 1191 T>C; 1546 C>G	51 G>A; 2141-2142delAA; 2296 C>T; 5684 T>C; 6692 C>G	Frameshift		None
*CYP2A6*21*	51 G>A; 1427 A>G	51 G>A; 6573 A>G	K476R		
*CYP2A6*22*	51 G>A; 474 C>G; 478 C>A	51 G>A; 1794 C>G; 1798 C>A	D158E; L160I		

determined.[18,27,29] In Asian populations, the allele frequencies of *CYP2A6*4* (11–20%), *CYP2A6*7* (4–7%), and *CYP2A6*9* (20%) were relatively high. The *CYP2A6*5* (~0.5%), *CYP2A6*6* (~0.4%), *CYP2A6*10* (0.5–1.1%), and *CYP2A6*11* (~0.7%) alleles were rare, and the *CYP2A6*2* allele was not found.[30,31] We found that cotinine formation was impaired in the homozygotes of either *CYP2A6*4*, *CYP2A6*7* and *CYP2A6*10* (**Fig. 2**).[18,19,27] In particular, cotinine was not detected in the plasma 2 hr after nicotine intake in subjects who are homozygotes of *CYP2A6*4*. *CYP2A6*9*, possessing a SNP in the TATA box, has been reported to decrease the transcriptional activity *in vitro*.[26] We also found that the *CYP2A6*9*

allele caused decreased expression levels of CYP2A6 mRNA and protein *in vitro*, and decreased the nicotine metabolism *in vivo*.[29]

In contrast to Asian populations, the *CYP2A6*2* allele, which lacks the enzymatic activity, has been found in Europeans, European-Americans, and African-Americans, although the allele frequencies are not so high (0.3–2.2%). *CYP2A6*9* has also been found in these populations with moderate frequencies (7.1–8.5%). Recently, we found novel alleles, *CYP2A6*17* (10.4%) and *CYP2A6*20* (1.6%), that are specific for the African-American population.[32,33] Since the *CYP2A6*17* allele (V365M) decreases the enzymatic activity and the *CYP2A6*20* allele (frameshift) lacks the activity, African-American subjects with these alleles showed low cotinine formation *in vivo*.[32,33] In summary, the large interindividual variability of cotinine formation from nicotine can be mostly explained by genetic polymorphisms of the *CYP2A6* gene. Smokers adapt their smoking behavior to maintain their plasma nicotine concentration.[2] The metabolism of nicotine to cotinine is the principal pathway by which active nicotine is removed from the circulation.[34] Associations between genetic polymorphisms of the *CYP2A6* gene and smoking behavior or the risk of lung cancer have also been suggested.[35–37]

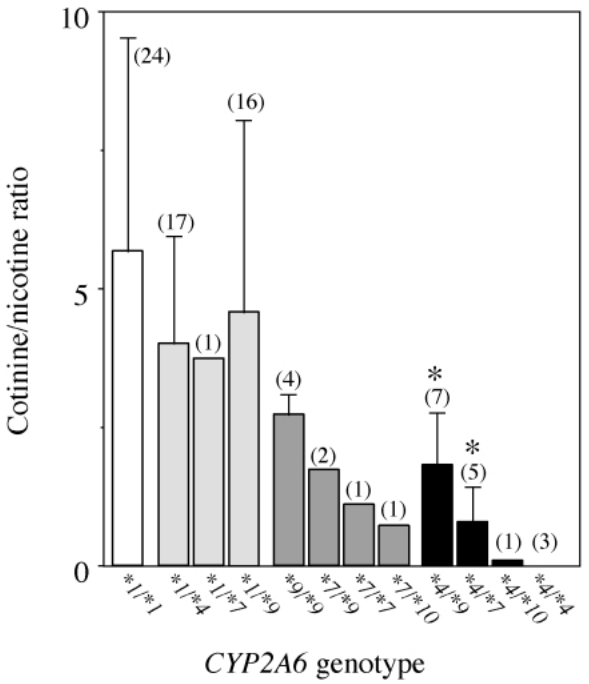

Fig. 2. Cotinine/nicotine ratios in Japanese subjects who were genotyped for *CYP2A6* alleles. The numbers of subjects are shown in parentheses. Data are expressed as mean ± SD. $^*P<0.05$, compared with *CYP2A6*1/CYP2A6*1*.

Effects of CYP2A6 Deletion on Comprehensive Nicotine Metabolism

In homozygotes of the *CYP2A6*4* allele, cotinine was not detected in the plasma 2 hr after nicotine intake.[18,19] The AUC values of cotinine in the subjects were prominently lower (one fifteenth) than those in subjects who possess the two active *CYP2A6* alleles (**Fig. 3**).[17] In homozygotes of the *CYP2A6*4* allele, the half-life of nicotine (2 hr) was prolonged to 11 hr.[17] To elucidate the nicotine metabolism in the subjects whose *CYP2A6* gene is deleted, the urinary excretion profile of nicotine metabolism was determined by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method.[38] In that study, 5 Japanese subjects who were genotyped for the *CYP2A6* gene participated. They chewed one piece of nicotine gum, and 24-hr accumulated urine samples were analyzed for nicotine metabolites. Out of 5 subjects, 3 subjects were genotyped as *CYP2A6*1A/CYP2A6*1A*, *CYP2A6*1A/CYP2A6*1B*, and *CYP2A6*1A/CYP2A6*4*. Since the *CYP2A6*1B* allele has a gene conversion with *CYP2A7* in the 3′-untranslated region,[24] it is considered that the enzymatic activity is the same as that of *CYP2A6*1A*.[24,29,39] Previously, we found that heterozygotes of the *CYP2A6*4* allele

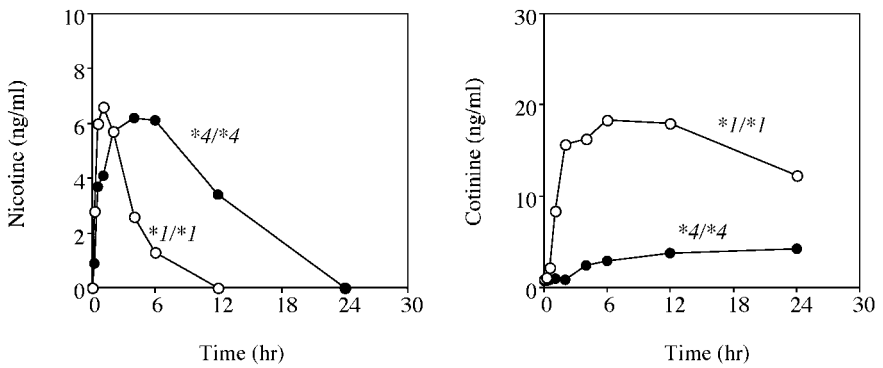

Fig. 3. Plasma concentrations of nicotine and cotinine in Japanese subjects who were genotyped as *CYP2A6*1/CYP2A6*1* or *CYP2A6*4/CYP2A6*4*. They chewed one piece of nicotine gum containing 2 mg of nicotine for 30 min.

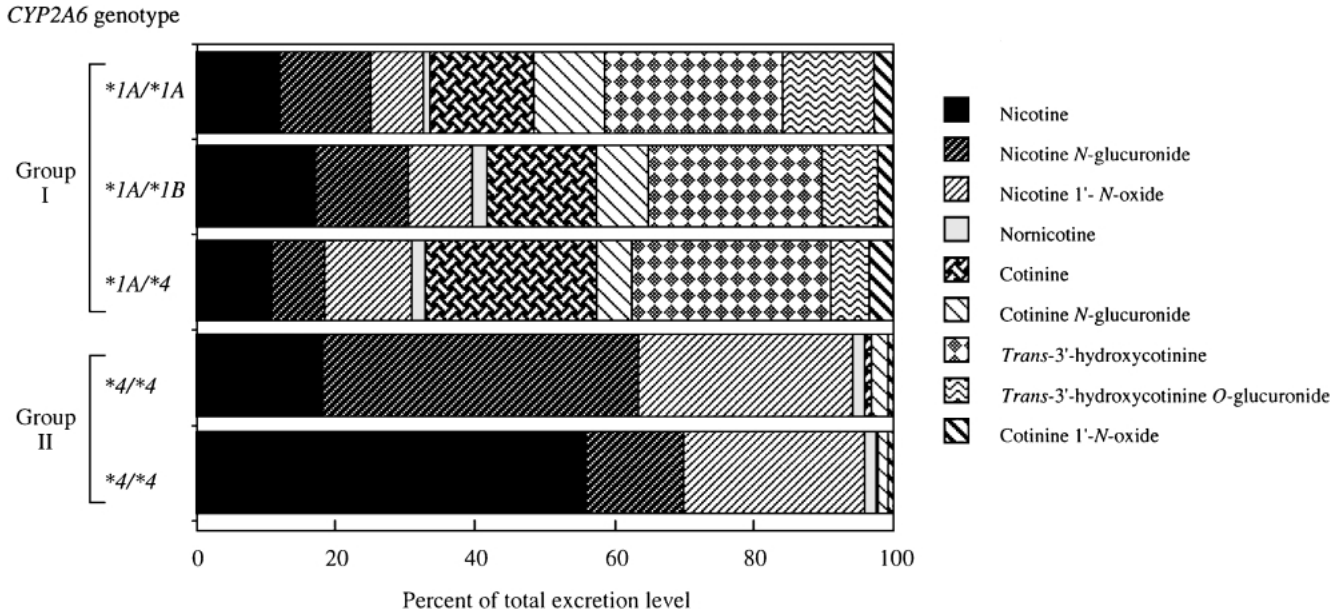

Fig. 4. Excretion levels of nicotine and its metabolites in 24-hr accumulated urine samples. *CYP2A6* genotypes in 5 subjects were determined. Three subjects genotyped as *CYP2A6*1A/CYP2A6*1A*, *CYP2A6*1A/CYP2A6*1B*, and *CYP2A6*1A/CYP2A6*4* were categorized as group I. Two subjects genotyped as *CYP2A6*4/CYP2A6*4* were categorized as group II. Norcotinine was not detected in any subjects.

can metabolize nicotine at levels similar to those of homozygotes of *CYP2A6*1A*.[18,19] Therefore, these 3 subjects were categorized as group I (two or one active allele). Two subjects who were genotyped as *CYP2A6*4/CYP2A6*4* were categorized as group II (none active allele).

Consistent with a previous report,[40] nicotine was mainly excreted as cotinine, *trans*-3′-hydroxycotinine, and their glucuronides in the 3 subjects of group I (**Fig. 4**). The sums of cotinine and cotinine-derived metabolites were 58.1–66.9% of the total excretion. The results support those of a previous report that 70–80% of nicotine is converted to cotinine.[7] In contrast, only trace levels of cotinine, cotinine *N*-glucuronide, and cotinine 1′-*N*-oxide were detected in the 2 subjects of group II, whereas *trans*-3′-hydroxycotinine and its *O*-glucuronide were not detected. It is considered that these results were due to the deletion of the entire *CYP2A6* gene in the 2 subjects. Although cotinine formation from nicotine is mainly catalyzed by CYP2A6, we previously found that CYP2B6 and CYP2D6 also possess trivial catalytic activity toward cotinine formation.[5] It has also been reported that the Km value of the cotinine formation by recombinant CYP2B6 was 10 fold higher than that by recombinant CYP2A6.[41] Thus, the cotinine would be formed compensatorily by these CYPs in the 2 subjects whose *CYP2A6* gene is deleted. The fact that no *trans*-3′-hydroxycotinine and its *O*-glucuronide could be detected in the subjects supports our previous data that CYP2A6 specifically catalyzes *trans*-3′-hydroxycotinine formation from cotinine.[6] In the 2 subjects of group II, the excretion levels of nicotine, nicotine *N*-glucuronide, and nicotine 1′-*N*-oxide were higher than those in the 3 subjects of group I. The sum of the excretion levels of nicotine and all metabolites were similar between group I (mean, 3,967 nmol) and group II (mean, 4,033 nmol). It has been reported that nicotine is absorbed by buccal (0.8 mg) and gut (0.06 mg) after the chewing of one piece of nicotine gum containing 2 mg of nicotine.[42] The expected absorbed nicotine of 0.86 mg corresponds to 5,306 nmol. Thus, it is assumed that most of the absorbed nicotine would be excreted in 24 hr, although the ingredients of the excreted metabolites were different between group I and group II. It was demonstrated that the metabolic profile of nicotine was affected by the deletion of CYP2A6, which is mainly responsible for nicotine metabolism.

Nicotine and Cotinine *N*-Glucuronidations

Glucuronidation is an important pathway of nicotine metabolism in humans. The average percentages of nicotine *N*-glucuronide and cotinine *N*-glucuronide excreted in smokers' urine were approximately 3–4% and 9–17% of the nicotine absorbed, respectively.[7,8,43] 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.[7]

Nicotine and cotinine *N*-glucuronidations in human

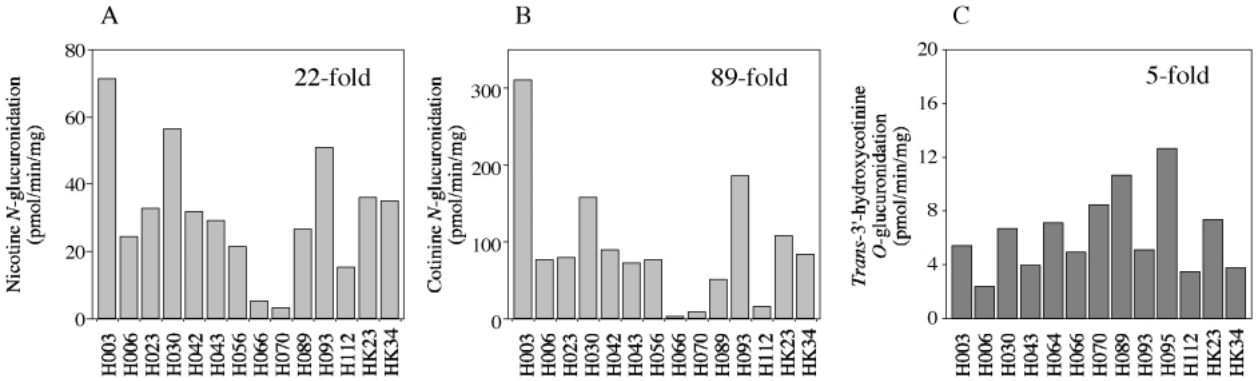

Fig. 5. Interindividual variability in nicotine *N*-glucuronidation (A), cotinine *N*-glucuronidation (B), and *trans*-3′-hydroxycotinine *O*-glucuronidation (C) in human liver microsomes. Human liver microsomes (0.25 mg/mL microsomal protein) were incubated with 2.5 mM UDP-glucuronic acid and 50 μM nicotine, 0.2 mM cotinine, or 1 mM *trans*-3′-hydroxycotinine at 37°C for 60 min. Each column represents the mean of duplicate determinations.

liver microsomes were characterized thoroughly in our study.[44] The kinetics of nicotine *N*-glucuronidation in human liver microsomes were clearly biphasic, whereas those of cotinine *N*-glucuronidation were monophasic. Based on the highly significant correlation between the nicotine *N*-glucuronidation and cotinine *N*-glucuronidation in human liver microsomes, the same UDP-glucuronosyltransferase (UGT) isoform(s) might be involved in these glucuronidations. We clarified that nicotine and cotinine *N*-glucuronidation are catalyzed mainly by UGT1A4 and partly by UGT1A9 with inhibition analyses and correlation analyses,[44] which were subsequently supported in a report by Kuehl and Murphy.[45]

Large interindividual variability in the nicotine *N*-glucuronidation (~22 fold) and cotinine *N*-glucuronidation (~89 fold) in human liver microsomes was observed (**Fig. 5**). In addition, Benowitz *et al*.[46] reported that the *in vivo* nicotine and cotinine *N*-glucuronidations appeared to be polymorphic in black subjects, although these were unimodal in white subjects. For all UGT isoforms, there are genetic polymorphisms. Recently, genetic polymorphisms of UGT1A4 that affect the enzymatic activity have been reported.[47–49] Therefore, genetic polymorphisms of the UGT1 isoforms might be one of the causal factors of the interindividual differences in nicotine and cotinine *N*-glucuronidation in humans. Furthermore, it has been reported that UGT1A9 is inducible by polycyclic aromatic hydrocarbons that are contained in cigarette smoke.[50] The inducibility of UGTs by cigarette smoke might affect the interindividual differences in nicotine and cotinine *N*-glucuronidations. Conclusive explanations for the interindividual and interethnic differences in the nicotine and cotinine *N*-glucuronidations remain to be found.

Trans-3′-hydroxycotinine *O*-Glucuronidation

Trans-3′-hydroxycotinine *O*-glucuronide is a major metabolite of nicotine in smokers' urine. Nicotine and cotinine are metabolized to *N*-glucuronide, whereas *trans*-3′-hydroxycotinine is metabolized to *O*-glucuronide *in vivo*. *N*-Linked glucuronide of *trans*-3′-hydroxycotinine was detected by incubation with human liver microsomes,[51,52] although it has never been detected in smokers' urine.[52,53] Since the *trans*-3′-hydroxycotinine *N*-glucuronidation was significantly correlated with the nicotine and cotinine *N*-glucuronidations, it may be catalyzed by UGT1A4.[51,52]

We characterized the *trans*-3′-hydroxycotinine *O*-glucuronidation in human liver microsomes and identified the human UGT isoform(s) involved in the glucuronidation. Recombinant UGT2B7 exhibited the highest *trans*-3′-hydroxycotinine *O*-glucuronosyltransferase activity, followed by 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.[54] *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 the morphine 3-glucuronosyltransferase activity catalyzed by recombinant UGT2B7 was also inhibited by imipramine, propofol, and androstanediol. In contrast, imipramine and androstanediol did not affect the *trans*-3′-hydroxycotinine *O*-glucuronosyltransferase activity catalyzed by recombinant UGT1A9. These results suggest that the

major UGT isoform involved in *trans*-3′-hydroxycotinine *O*-glucuronidation in human liver microsomes would be UGT2B7. Although morphine glucuronidation is catalyzed by UGT2B7,[55] 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. Tobacco specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, has been reported to be metabolized to its *O*-glucuronide by UGT1A9 and UGT2B7[56] and its *N*-glucuronide by UGT1A4.[57] 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.

Interindividual variability in the *trans*-3′-hydroxycotinine *O*-glucuronosyltransferase activities in microsomes from 13 human livers was at most 5 fold **(Fig. 5)**. It was not so large, compared with those in the nicotine and cotinine *N*-glucuronidation in the panel of human liver microsomes. The results suggest that the interindividual variability of the expression level or activity of UGT2B7 might be lower than those of other UGT isoforms. Alternatively, the involvement of multiple enzymes in the *trans*-3′-hydroxycotinine *O*-glucuronosyltransferase might reduce the interindividual variability.

Conclusion

Variability in nicotine metabolism could be an important determinant of nicotine clearance. Recent advances in nicotine metabolism research have elucidated some of the causes for the large interindividual differences in metabolic capacity. Special emphasis is given to the effects of the genetic polymorphisms of *CYP2A6* gene in relation to the interindividual differences in the nicotine metabolism to cotinine. Interindividual variability has been also observed in the glucuronidation pathways of nicotine and its metabolites. The identification of UGT isoforms involved in the glucuronidations could be helpful for the consideration of such interindividual variability. The causes of the interindividual and interethnic differences in the glucuronidations of nicotine and its metabolites, in relation to the genetic polymorphisms of UGTs or regulation of their expressions, will be studied in the future.

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