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The influence of CYP2A6 polymorphisms and cadmium on nicotine metabolism in Thai population

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ABSTRACT

We investigated the influence of genetic, cadmium exposure and smoking status, on cytochrome P450-mediated nicotine metabolism (CYP2A6) in 182 Thai subjects after receiving 2 mg of nicotine gum chewing for 30 min. The urinary excretion of cotinine was normally distributed over a 2 h period (logarithmically transformed). Individuals with urinary cotinine levels in the ranges of 0.01–0.21, and 0.52–94.99 μg/2 h were categorized as poor metabolizes (PMs: 6.5%), and extensive metabolizers (EMs: 93.5%), respectively. The majority of EMs (45%) carried homozygous wild-type genotypes (*CYP2A6*1A/*1A*, *CYP2A6*1A/*1B* and *CYP2A6*1B/*1B*), whereas only 1% of PMs carried these genotypes. Markedly higher frequencies of EMs were also observed in all heterozygous defective genotypes including the null genotype (*4C/*4C; 1 subject).

A weak but significant positive correlation was observed between total amounts of urinary cadmium excretion and total cotinine excretion over 2 h. Our study shows generally good agreement between CYP2A6 genotypes and phenotypes. Smokers accumulated about 3–4-fold higher mean total amounts of 2-h urinary cadmium excretion (127.5 ± 218.2 ng/2 h) than that of non-smokers (40.5 ± 78.4 ng/2 h). Among the smokers ($n = 16$), homologous wild-type genotype *1/*1 was significantly the predominant genotype (6/16) compared with other defective allele including *4C/*4C. In addition, 2 h urinary excretion of cotinine in smokers of all genotypes was significantly higher than non-smokers. The proportion of smokers who smoked more than 5 cigarettes/day was significantly higher in EMs in all CYP2A6 genotypes ($n = 14$) than in PMs ($n = 0$).

1. Introduction

Cytochrome P450 2A6 (CYP2A6), known as nicotine *C*-oxidase plays important role in the metabolism of several xenobiotics including nicotine and coumarin (Oscarson, 2001; Satarug et al., 2003; Oscarson et al., 1998; Pelkonen et al., 2000; Raunio et al., 2001). Hepatic CYP2A6 activity varies widely among healthy individuals as well as patients with liver disease (Satarug et al., 1996; Ujjin et al., 2002). Human hepatic CYP2A6 activity may be phenotypically categorized as poor metabolizers (PMs), intermediate metabolizers (IMs), and extensive metabolizers (EMs). A number of allelic variants of the *CYP2A6* gene have been reported. The frequency distributions of the *CYP2A6* alleles show remarkable differences among ethnic groups. The *CYP2A6*1A* and **1B* alleles both encode for the wild-type CYP2A6.1 protein, but possess different 3′-UTR sequences. The *CYP2A6*4* is a null allele as a result of whole gene deletion (Ariyoshi et al., 2001). The *CYP2A6*7* and *CYP2A6*8* alleles, which are found only in the **1B* allele, contain single nucleotide polymorphisms (SNPs), resulting in amino acid substitutions (Ariyoshi et al., 2001). *CYP2A6*10* is a haplotype containing both the non-synonymous SNPs found in *CYP2A6*7* and *CYP2A6*8*. The *CYP2A6*9* allele contains a mutation in the TATA box in the proximal promoter (−48T > G) (Pitarque et al., 2001). There is evidence that individuals with the *CYP2A6* gene deficiency (homozygous for null allele, *CYP2A6*4/*4* genotype), and thus lacking the ability to metabolize nicotine, are associated with a decreased likelihood of being a smoker and a decrease in cigarette consumption (Satarug et al., 2006). Individuals with other defective *CYP2A6* alleles including *CYP2A6*7*, *CYP2A6*8* and *CYP2A6*10*, who have a decreased ability to metabolize nicotine, are unlikely

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to become nicotine dependent. Therefore, the possibility of altering of CYP2A6 enzyme activity and expression (with chemical inhibitors or CYP2A6 enzyme knockout) may serve as a selective means to pharmacologically intervene smoking behavior since CYP2A6 is responsible for approximately 90% of nicotine oxidation.

Drugs and structurally unrelated compounds such as phenobarbital, rifampicin, clofibrate, pyrazole, thioacetamide, griseofulvin, and porphyrinogenic agents (Hahnemann et al., 1992; Salonpää et al., 1995), as well as heavy metals including lead, tin, cobalt, and in particular, cadmium (Abu-Bakar et al., 2007; Emde et al., 1996; Oscarson, 2001; Pelkonen et al., 2006; Raunio et al., 2001; Satarug et al., 2000, 2004a,b,c; Satarug and Moore, 2004), have been reported as inducers of CYP2A5 (the murine orthologue of human CYP2A6) and of CYP2A6 in human hepatocyte primary cultures. This suggests that the variability in CYP2A6 phenotypes may be due primarily to CYP2A6 genetic polymorphisms, and coupled with exposure to CYP2A6 inducers or repressors in some individuals. Cadmium found in tobacco smoke and food is known to accumulate in the tissues where CYP enzymes are found, and variations in the expression of selected CYP proteins in liver and kidney may be attributable to human exposure to environmental cadmium. Evidence for the potential effects of environmental cadmium on the expression of CYP in human livers first arose when a positive association between hepatic cadmium and CYP content was demonstrated in humans (Baker et al., 2001).

The objective of the present study was to investigate the CYP2A6 phenotypes (CYP2A6-mediated nicotine metabolism) and their association with genotypes in a Thai population using nicotine as a probe substrate. In addition, influence of cadmium exposure including from tobacco smoking, on CYP2A6 genotypic expression was also investigated.

2. Materials and methods

2.1. Chemicals

Cotinine, acetaminophen (internal standard), 7-hydroxycoumarin, 7-amino-4-methyl-coumarin (internal standard), and β-glucuronidase were purchased from Sigma Chemicals Co. Ltd. (St. Louis, MO, USA). All organic solvents used for analyzes of coumarin, 7-hydroxycoumarin and cotinine were HPLC-grade quality (LAB-Scan Ltd. (Analytical Sciences, Thailand). Takara LA Taq DNA polymerase was purchased from Takara (Kyoto, Japan) and AmpliTaq DNA polymerase was obtained from Applied Biosystems. Restriction enzymes and a 100 bp DNA ladder were purchased from New England Biolabs (Beverly, MA, USA). Proteinase K was obtained from Promega (WI, USA).

2.2. Study subjects

A total of 182 unrelated, healthy adult Thai subjects of both genders (89 males, 93 females; 28 smokers, 154 non-smokers) aged between 18 and 50 years and weighing between 42 and 68 kg, were included in this study. The study was part of a recent report on the investigation of CYP2A6 genotypes and coumarin-oxidation phenotypes in a Thai population (Mahavorasirikul et al., 2009), where subjects were randomly allocated to receive nicotine gum and coumarin on two separate occasions at least 1 week apart to avoid carry over of nicotine or coumarin. Participants were residents of the three main areas of Thailand, *i.e.*, Pathumtani Province (central region; $n = 58$), Khon Kaen Province (northeastern region; 39), and Tak Province (northern region; $n = 85$). The study protocol was approved by the Ethics Committees of the Faculty of Medicine, Khon Kaen University, and the Ministry of Public Health of Thailand. Written informed consents for study participation were obtained from all subjects who had been informed of the study protocol. All subjects were healthy as verified by clinical and laboratory assessments. Exclusion criteria included those who had used medication within 2 days prior to the start of the experimental period. Smokers were asked to refrain from smoking 3 days before the study. The ethnic background, full medication history, history of pregnancy, smoking and alcohol consumption, specific food ingredient consumption, and any history of drug use were ascertained by means of a questionnaire interview. The subjects recruited in the study were students, hospital staff, and farmers; there was no occupational exposure to cadmium in all subjects.

2.3. Phenotyping of in vivo nicotine metabolism

Following overnight fasting, subjects were given half a piece (2 mg) of nicotine gum (Nicorette™, 4 mg nicotine gum), and were asked to chew the gum at the rate of 15 cycles/min for 30 min, followed by 300 ml drinking water. Urine samples were collected during a 2-h period after nicotine intake. Total volumes of urine were recorded and an aliquot of 5 ml was stored at −20 °C for measurement of cotinine (the metabolite of nicotine) concentrations using high performance liquid chromatography–tandem mass spectrometry (LC/MS/MS) (Xu et al., 2004). The phenotype classification of CYP2A6 activity was determined by probit analysis.

2.4. Genotyping of CYP2A6

For genotyping of *CYP2A6*, 1 ml of venous blood was collected from all volunteers into Lithium-EDTA tubes. Genomic DNA (gDNA) was extracted from the buffy coat using a standard phenol–chloroform extraction technique. The CYP2A6 genotypes were examined using restriction fragment length polymorphism (PCR-RFLP), allele-specific two-step PCR, and real-time PCR techniques as described in detail in the previous report (Mahavorasirikul et al., 2009).

2.5. Determination of urinary cadmium excretion

Total amounts of urinary cadmium excreted over 2 h (μg/g creatinine) were used as an index of long-term cadmium exposure. Total volumes of each urine collection were recorded and a 5 ml portion was aliquoted into a 10 ml polypropylene tube and stored at −20 °C until required. Urinary concentrations of cadmium were measured using electrothermal (graphite furnace) atomic absorption spectrometry (GFAAS) (Kendüzler and Türker, 2005).

2.6. Data and statistical analysis

Statistical analysis was performed using SPSS version 12.0. (Lead Technologies, Inc.). Associations between measured variables were analyzed by using the Pearson's correlation test. One-way ANOVA was used to explore statistically significant differences in mean values of test variables in three groups. The Student's *t*-test was used to determine statistically significant difference between two groups. Comparison of qualitative data between two groups (*i.e.*, proportion) was done by Chi-square test. Statistically significant difference was set at $\alpha = 0.05$ for all tests.

3. Results

3.1. Urinary cotinine

Fig. 1 shows the frequency distribution of the amounts of cotinine (logarithmically transformed) excreted in urine over 2 h in the sample population of 154 subjects who were non-smokers. Individuals with 2 h urinary cotinine excretion in the ranges of 0.01–0.21 (6.5%) and 0.52–94.99 (93.5%) μg/2 h were classified as poor metabolizers (PMs), and extensive metabolizers (EMs), respectively. The mean total amount of urinary cotinine excretion in 2 h was significantly lower in PMs (1.03 ± 0.71 μg/2 h) compared with EMs (32.57 ± 22.24 μg/2 h) ($p < 0.0001$). Female subjects in all metabolic status groups (PM, EM and combined data) had significantly lower

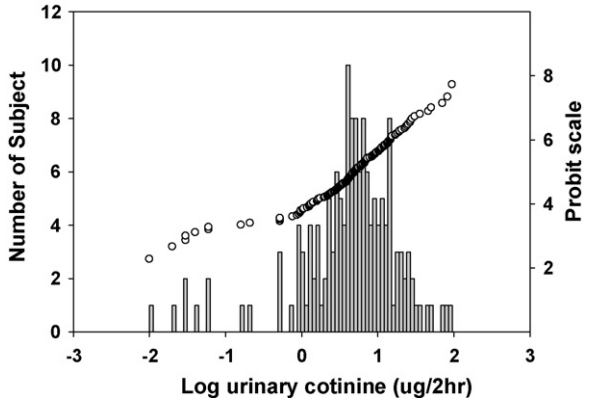

Fig. 1. Frequency distribution of total amounts of cotinine (logarithmically transformed) excreted in urine over 2 h (μg/2 h) in a total of 134 Thai healthy subjects who were non-smokers.