Effect of age and smoking on in vivo CYP1A2, flavin-containing monooxygenase, and xanthine oxidase activities in Koreans: Determination by caffeine metabolism

Objectives: To assess the effect of gender, age, and smoking habits on the in vivo activities of CYP1A2, flavin-containing monooxygenase (FMO), and xanthine oxidase in Korean subjects.
Methods: One hundred thirty-three age- and gender-matched healthy Korean volunteers (age range, 21 to 78 years; mean age, 35.3 ± 16.6 years) with and without smoking habits participated. After drinking a cup of coffee (200 mL) that contained 110 mg caffeine, a 1-hour urine sample (between 4 and 5 hours) was collected and caffeine metabolites were analyzed by HPLC.
Results: There were marked individual variations in CYP1A2 [(1,7-dimethylurate + paraxanthine)/caffeine], FMO (theobromine/caffeine), and xanthine oxidase (1-methylurate/1-methylxanthine) activities (14-, 42-, and 9-fold, respectively). However, the mean values of these enzyme activities in the nonsmokers were not different between men and women. In the nonsmoking subjects in their 20s, the mean values of CYP1A2 and FMO activities (13.5 ± 5.9 and 2.1 ± 1.9, respectively) were higher than those (7.9 ± 1.8 and 0.95 ± 0.22) of older decennial age groups. Xanthine oxidase activities were the same for all age groups (subjects in their 20s through their 70s). CYP1A2 activity of the smokers (20.0 ± 9.6) was higher than that of the nonsmokers (10.8 ± 5.8; $P < .001$). Similarly, the FMO activity in smokers (3.4 ± 2.7) was higher than that of the nonsmokers (1.8 ± 1.7; $P < .001$). The xanthine oxidase activity (1.3 ± 0.5) was not increased in smokers (1.4 ± 0.5; $P = .46$).
Conclusions: Results of this caffeine metabolism study conducted with age- and gender-matched healthy Korean volunteers with and without smoking habits provided the baseline and the widely varying interindividual activities of CYP1A2, FMO, and xanthine oxidase in a Korean population. The results also suggested that drugs metabolized by CYP1A2 and FMO may require individualized dose adjustment according to the age and smoking habits of the subjects. (Clin Pharmacol Ther 2000;67:258-66.)

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Caffeine is a widely consumed trimethylxanthine derivative alkaloid that is contained in beverages such as coffee, tea, and cola. Caffeine undergoes extensive oxidative metabolism in humans, initially by *N*-demethylation to theobromine, paraxanthine, and theophylline and subsequently to 1,7-dimethylurate (17U), 1-methylxanthine (1X), and 1-methylurate (1U).[1] All of these primary and secondary metabolites of caffeine are excreted in urine and are easily detectable. Because the productions of these metabolites are known to be catalyzed by combined actions of CYP1A2 and xanthine oxidase, caffeine has been used as a noninvasive probe for determination of in vivo CYP1A2 and xanthine oxidase activities in humans.[2-5]

CYP1A2 is known to catalyze the oxidative metabolic activation of various procarcinogens such as aromatic amines and heterocyclic amines that cause colorectal or bladder cancers in humans.[6] In addition, CYP1A2 is known to be involved in the metabolism of various clin-

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Table I. Demographic characteristics of the volunteers

Age (y)	*20-29*	*30-39*	*40-49*	*50-59*	*60-69*	*70-79*	*Total*
Men	56	11	4	4	6	3	84
Smokers	27	3	1	2	4	1	38
Nonsmokers	29	8	3	2	2	2	46
Women	20	5	6	7	8	3	49
Smokers	—	—	—	—	—	—	0
Nonsmokers	20	5	6	7	8	3	49
TOTAL	76	16	10	11	14	6	133

ically useful drugs such as tacrine (used in patients with Alzheimer's disease)[7] and brofaromine (a selective inhibitor of monoamine oxidase A used in aged patients with mental disease).[8] Long-term use of these drugs in elderly patients with mental disease is known to produce hepatotoxicity. Furthermore, CYP1A2 is known to be inducible by the polyaromatic hydrocarbons and halogenated hydrocarbons contained in cigarette smoke.[3] Information on the effects of age and smoking on CYP1A2 activity would therefore provide useful guidelines on cancer susceptibility, drug toxicity, and drug interactions.

Together with CYPs, the flavin-containing monooxygenases (FMOs) are known to oxidize various endogenous and exogenous chemicals.[9] FMOs oxidize many clinically used drugs that contain the *N, S,* and *P* atoms, such as cimetidine, methimazole (INN, thiamazole), ranitidine, arylamines, *N*-methylaniline, thiocarbamides, aminothiols, and a variety of pyschoactive drugs to their respective oxides.[10]

Physiologically, FMO is known to oxidize trimethylamine, the volatile fish-smelling metabolite that arises from diets that contain choline, to trimethylamine *N*-oxide, which is excreted in urine without any smell. Therefore subjects who lack FMO activity and cannot convert trimethylamine to its *N*-oxide are known to have trimethylaminuria (fish-odor syndrome).[11]

In previous studies, we found that production of theobromine from caffeine was catalyzed primarily by FMO.[12,13] We have developed a noninvasive method of phenotyping the in vivo FMO activity by assessing the urinary molar concentration ratio of theobromine/caffeine,[14] and we used this newly developed FMO phenotyping method to identify several mutations that are present in the *FMO3* gene in a Korean population.[15] Information on the individual in vivo FMO activity determined by the "coffee test" would therefore aid in choosing proper dose of a given drug that is metabolized by FMO.

Xanthine oxidase is responsible for the oxidation of several endogenous compounds, such as hypoxanthine and xanthine, as well as some important cancer chemotherapeutic drugs such as mercaptopurine and azathioprine.[5] In particular, xanthine oxidase eventually oxidizes xanthine and caffeine to their respective uric acids, with a release of reactive oxygen species that cause cellular necrosis. Increased production of reactive oxygen species by the elevated xanthine oxidase activity in chronic inflammatory diseases is known to cause serious disease in various organs, such as a lung with chronic pulmonary obstruction that occurs with long-term smoking.[16] Information on the effect of age and smoking on the in vivo xanthine oxidase activity would therefore provide useful information on the toxic and necrotic effects of drugs that occur by interaction with reactive oxygen species.

CYP1A2, FMO, and xanthine oxidase play important roles in the metabolism of many endogenous and foreign chemicals and drugs. It would therefore be of interest to learn whether the in vivo activities of these enzymes are modulated by age and smoking habits. In this study, we determined the effect of age and smoking on CYP1A2, FMO, and xanthine oxidase activities in Korean subjects by assessing the molar concentration ratios of urinary caffeine metabolites after consumption of a cup of coffee.

METHODS

Study protocol. Age-matched healthy Korean volunteers were recruited from students and personnel at Inha Medical College (82) and elderly people living near the Inha university (51) located at Inchon, an urban city. Volunteers included 84 men (39 cigarette smokers and 45 nonsmokers) and 49 women (all nonsmokers). Their ages ranged from 21 to 78 years (mean ± SD, 35.3 ± 16.6 years), and their body weights ranged from 42 to 95 kg (mean ± SD, 60.2 ± 11.1 kg). Detailed demographic characteristics of the participants are summarized in Table I. All 133 native Korean volunteers who were allowed to participate in this study provided written informed consent and were judged to be healthy

Table II. Comparisons of CYP1A2, flavin-containing monooxygenase (FMO), and xanthine oxidase activities in the nonsmoking men (n = 46) and women (n = 49)

	Range	*Median*	*Mean ± SD*	*P Value**
CYP1A2				
Men	2.5-29.3	9.9	11.1 ± 6.0	
Women	2.1-22.0	9.7	10.5 ± 5.7	.67
FMO				
Men	0.23-8.59	1.43	2.00 ± 1.83	
Women	0.21-8.76	1.18	1.53 ± 1.47	.17
Xanthine oxidase				
Men	0.52-2.74	1.23	1.30 ± 0.51	
Women	0.42-3.79	1.26	1.30 ± 0.52	.99

*Mean values of enzyme activities obtained from corresponding men and women are compared with use of the Mann-Whitney test, and their *P* values are indicated.

according to medical history, physical examination, and routine laboratory analysis. None of the subjects were using any concurrent medications. This study was conducted in accordance with the Korean Guidelines for Good Clinical Practice, and the protocol was approved by the Institutional Review Board of Inha University Hospital (Inchon, Korea). We did not control for the menstrual cycle of female volunteers. All volunteers were asked to not take any methylxanthine-containing drinks, foods, or drugs for 2 days before and during the study period. Compliance was confirmed by detection of no caffeine in the control baseline urine.

Urine collection. After an overnight fast (10 hours), each volunteer voided a control baseline urine sample and was given a cup of coffee (200 mL) prepared from two packs of instant coffee (Taster's Choice, 12 g, Nestle, Vevey, Switzerland) that contained 110 mg (55.1 ± 1.8 mg/pack) caffeine as determined in our laboratory. A 1-hour urine sample was collected between 4 and 5 hours after the coffee intake, and the volume was measured. Immediately thereafter the pH of all urine samples was adjusted to 3.5 with hydrochloric acid as described by Nakajima et al.[17] A 10-mL aliquot was stored at –80°C for HPLC analysis and the remainder was discarded.

HPLC analysis of caffeine and its metabolites in urine samples. After thawing the frozen aliquot, 500 μL urine was mixed with 250 mg ammonium sulfate for 2 minutes to precipitate proteins that may have been present. Subsequently, 24 μg 4-acetamidophenol (internal standard) dissolved in a solvent mixture composed of chloroform and isopropanol (4:1; vol/vol) was mixed and centrifuged for 5 minutes at 1400*g*. The organic solvent layer (5.5 mL) was collected by suction and was concentrated by vacuum centrifugation for 90 minutes at 45°C. The concentrated residue was then reconstituted to 800 μL with 0.05% acetic acid and filtered through a 0.45-μm pore size filter (Elutip-D, Schleicher & Schuell, Dassel, Germany). One hundred microliters of the filtrate was injected onto an HPLC column. Caffeine and its metabolites were analyzed by use of an HPLC system according to the method described by Chung and Cha.[12]

Data analysis. The CYP1A2 activity was assessed by use of the molar concentration ratio of 17U plus paraxanthine to caffeine, (17U + paraxanthine)/caffeine, as described by Butler et al.[2] The FMO activity was assessed by the ratio of theobromine to caffeine (theobromine/caffeine) as described previously,[14,15] and the xanthine oxidase activity was assessed by the ratio of 1U to 1X (IU/IX).[2] Because the baseline control urine did not contain any measurable amounts of caffeine, 17U, paraxanthine, theobromine, 1U, or 1X, participants were believed to have complied with the request to abstain from methylxanthine-containing drinks, foods, or drugs, and urine samples collected after the administration of coffee were suitable for assessment of CYP1A2, FMO, and xanthine oxidase activities. To determine whether the activities of these enzymes were affected by factors such as age, smoking, and gender, statistical parameters obtained from these groups were compared by use of the Mann-Whitney test.

RESULTS

Lack of gender-related differences in CYP1A2, FMO, and xanthine oxidase activities. As shown by the demographic characterstics of the volunteers (Table I), there were 46 nonsmokers and 38 smokers among the 84 male volunteers, and all of the 49 female volunteers were nonsmokers. Therefore to compare gender-related differences of CYP1A2, FMO, and xanthine oxidase activities, only results obtained from the nonsmoking male and female subjects were compared (Table II). The range of CYP1A2 activities (as assessed

Table III. Caffeine metabolites in 1-hour (between 4 and 5 hours) urine samples obtained from nonsmokers and smokers after coffee intake

	Nonsmokers (n = 94)	*Smokers (n = 39)*	*P Value**
1,7-Dimethylurate + paraxanthine (μmol/L)	256.7 ± 152.1	304.0 ± 103.3 (118%)†	.008
Theobromine (μmol/L)	34.9 ± 38.4	43.9 ± 46.9 (126%)†	.050
Caffeine (μmol/L)	26.3 ± 20.6	18.6 ± 8.6 (70%)†	.004

Smoking increased not only 1,7-dimethylurate + paraxanthine (a numerator fraction for CYP1A2 activity) but also theobromine (a numerator fraction for FMO activity) and indicated that smoking induced the CYP1A2 and FMO activities in humans.

*The mean values of urinary caffeine metabolite concentrations of nonsmokers and smokers are compared by use of the Mann-Whitney test, and their *P* values are indicated.

†Percentage value in parentheses indicates the percentage compared with that of the nonsmokers.

by the urinary ratio of [17U + paraxanthine]/caffeine) for all ages of the nonsmoking men was between 2.5 to 29.3, showing 12-fold individual differences, and mean value was 11.1 ± 6.0. The range for all nonsmoking women was between 2.1 and 22.0, a 10-fold individual difference, and mean value was 10.5 ± 5.7. The CYP1A2 activities of nonsmoking male and female volunteers were not significantly different ($P = .67$).

The range of FMO activities (urinary theobromine/caffeine) for all ages of nonsmoking male volunteers was between 0.23 and 8.59, showing 37-fold maximal individual differences. For the nonsmoking female volunteers, the lowest FMO activity was 0.21 and the highest was 8.76, showing 42-fold maximal individual differences. The mean FMO activity was 2.00 ± 1.83 for men and 1.53 ± 1.47 for women. However, the mean values for FMO activity were not significantly different ($P = .17$).

The xanthine oxidase activities (1U/1X) for all ages of nonsmoking male and female volunteers ranged between 0.52 and 2.74 and 0.42 and 3.79, respectively. Their mean values were the same (1.30 ± 0.51) and were not significantly different ($P = .99$) (Table II). We therefore concluded that gender does not have any influence on the activities of CYP1A2, FMO, and xanthine oxidase in a Korean population.

Effect of smoking on the amounts of 17U plus paraxanthine, theobromine, and caffeine in urine. In assessing the in vivo CYP1A2, FMO, and xanthine oxidase activities, we used the urinary molar concentration ratios of (17U + paraxanthine)/caffeine, theobromine/caffeine, and 1U/1X, respectively. 17U, paraxanthine, and theobromine are produced from combined and interdependent catalytic actions of CYP1A2 and FMO. If the activities of these enzymes are enhanced by smoking, production of these metabolites would be increased at the expense of caffeine. This would reduce the amount of caffeine excreted in urine. Use of urinary concentration ratios to determine enzyme activities results in larger than the actual ("apparent") CYP1A2 or FMO activity values. Results shown in Table III indicate that urinary concentrations of both 17U + paraxanthine and theobromine were increased significantly in the smokers. In addition, the concentration of caffeine was decreased significantly. This indicated that the activities of both CYP1A2 and FMO obtained by urinary concentration ratios of (17U + paraxanthine)/caffeine and theobromine/caffeine were increased by smoking.

Effect of smoking on the apparent CYP1A2, FMO, and xanthine oxidase activities. To evaluate the effect of smoking on CYP1A2 activities, the (17U + paraxanthine)/caffeine ratio values obtained from 95 nonsmokers (46 men and 49 women) and 38 male smokers were compared. The statistical mean of CYP1A2 activities was 10.78 ± 5.79 for nonsmokers (men and women) and 20.00 ± 9.57 for male smokers, which was significantly different ($P < .001$). Therefore the apparent CYP1A2 activity was increased by smoking. When the individual CYP1A2 activities of smokers and nonsmokers were compared in the frequency distribution plot (Fig 1, *A*), the distribution of smokers was shifted toward higher ratios and showed that CYP1A2 activity was increased.

FMO activities (theobromine/caffeine) of 95 nonsmokers and 38 smokers were compared. The mean of the ratios was 1.76 ± 1.66 in the nonsmokers and 3.40 ± 2.72 in the smokers, which was significantly different ($P < .001$). Similarly, when the individual FMO activities of smokers and nonsmokers were compared in the frequency distribution plot (Fig 1, *B*), the distribution of smokers was again shifted toward higher ratios, indicating that the FMO activity was increased.

Xanthine oxidase activities (1U/1X) of 95 nonsmokers and 38 smokers were compared, and the mean of the 1U/1X ratios obtained was 1.37 ± 0.47 for the nonsmokers and 1.30 ± 0.51 for the smokers. Unlike the CYP1A2 and FMO activities, the xanthine oxidase activities were not different ($P = .46$) between smokers and nonsmokers. The frequency distribution of xanthine

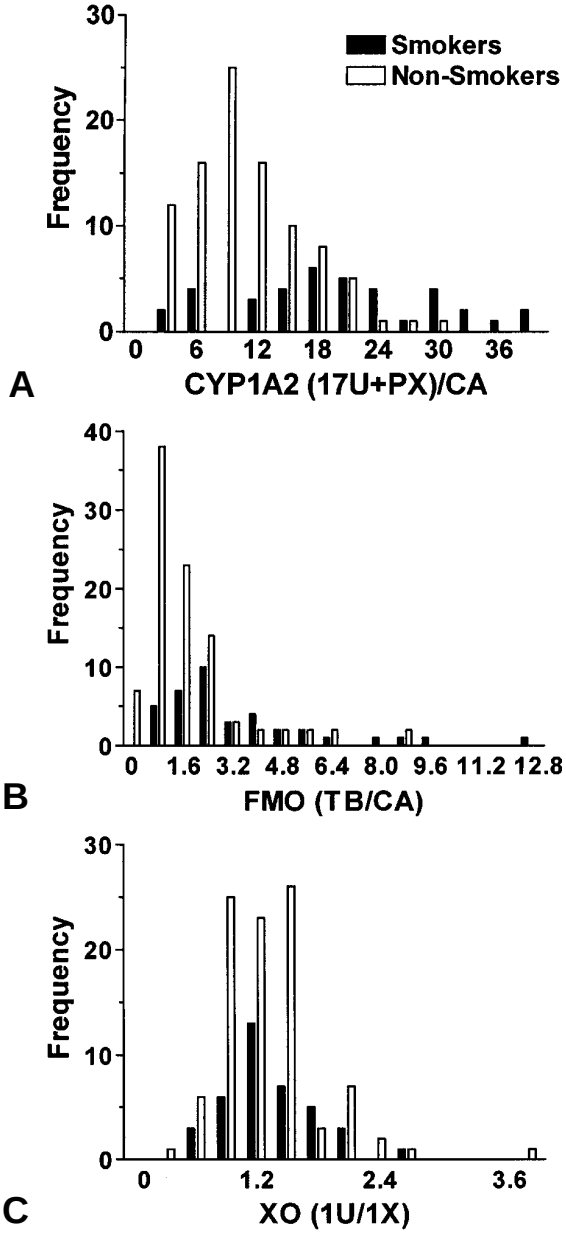

Fig 1. Effect of smoking on the distribution of CYP1A2 **(A),** flavin-containing monooxygenase (FMO; **B**), and xanthine oxidase (XO; **C**) activities. The frequency distribution plots of CYP1A2 **(A)** and FMO **(B)** activities of smokers are shifted toward higher (17U + paraxanthine)/caffeine and theobromine/caffeine ratios compared with those of the nonsmokers. The mean of the (17U + paraxanthine)/caffeine ratio was 10.8 ± 5.7 for nonsmokers and 20.0 ± 9.6 for smokers ($P < .001$). The mean of the theobromine/caffeine ratio was 1.76 ± 1.66 for nonsmokers and 3.40 ± 2.72 for smokers ($P < .001$). However, the frequency distribution plots of xanthine oxidase activities **(C)** of nonsmokers (1.30 ± 0.51) and smokers (1.37 ± 0.47) were almost matched.

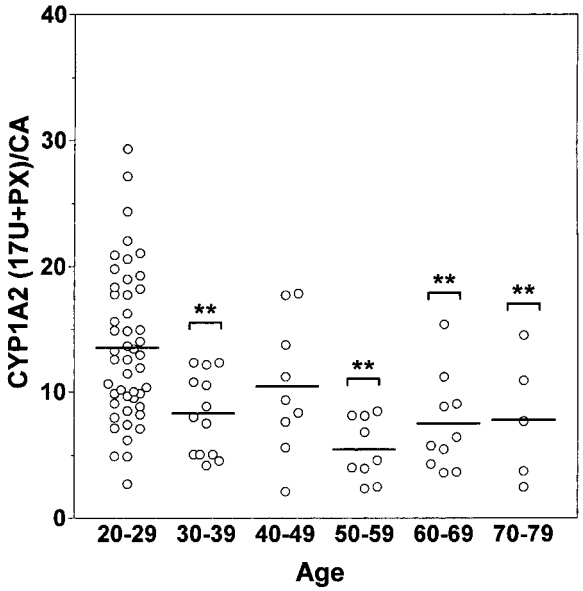

Fig 2. Effect of age on the CYP1A2 activity of nonsmokers. The mean CYP1A2 activity of the subjects in their 20s was the highest and was higher than those of the subjects in their 30s, 50s, 60s, and 70s (**$P < .01$).

oxidase activities for the smokers was not shifted from that of the nonsmokers (Fig 1, *C*) and indicated that xanthine oxidase activity was not increased by smoking.

Effect of age on CYP1A2, FMO, and xanthine oxidase activities. The mean of CYP1A2 activities in the nonsmoking subjects (men and women combined) in their 20s (n = 49) was 13.5 ± 5.9. The mean CYP1A2 activity of the decennial group of subjects in their 30s was 8.2 ± 3.2 and was only 60.7% ($P < .01$) of the activity found in the group of subjects in their 20s. The enzyme activities of the decennial groups of subjects in their 40s, 50s, 60s, and 70s were only 77% ($P = .15$), 41% ($P < .01$), 55% ($P < .01$), and 59% ($P < .01$), respectively, of the enzyme activities of the group in their 20s (Fig 2). The enzyme activities found in these older decennial age groups were not significantly different from each other.

The mean of FMO activities in the nonsmoking subjects (men and women) in their 20s was 2.5 ± 1.9. As with the CYP1A2 activity, the FMO activity observed in the group of sujbects in their 20s was the highest among all decennial age groups compared (Fig 3). Although the data are not shown, the FMO activities of nonsmoking male and female subjects in each of the decennial age groups were not significantly different. The mean of FMO activities in the group of subjects in their 20s was 2.5 ± 1.9 and the mean of the group of

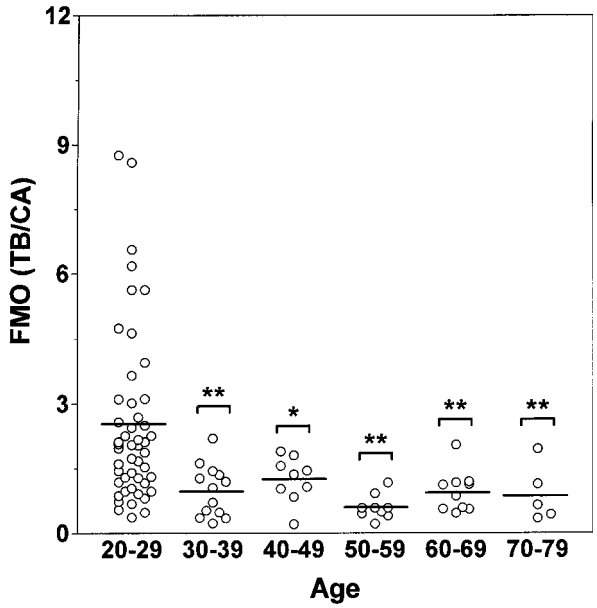

Fig 3. Effect of age on the FMO activity of nonsmokers. The mean FMO activity of the subjects in their 20s was the highest and was higher than those of the subjects in their 30s, 40s, 50s, 60s, and 70s (*P < .05; **P < .01).

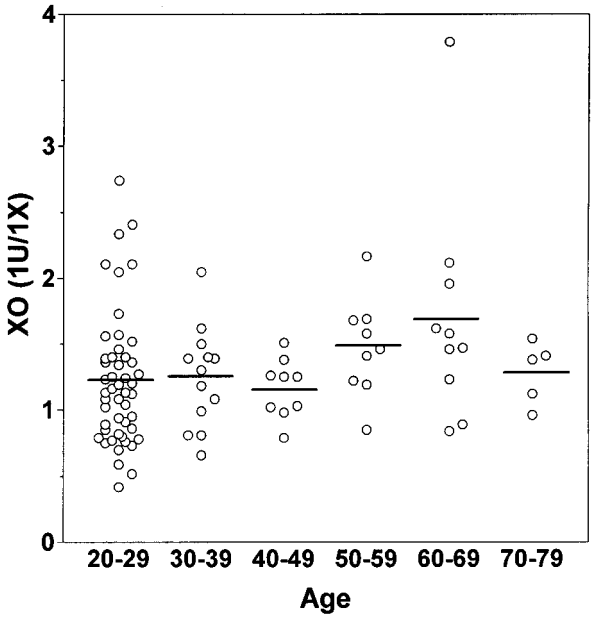

Fig 4. Effect of age on the xanthine oxidase activity of nonsmokers. The mean xanthine oxidase activity did not change with age. However, the xanthine oxidase activity appeared to increased gradually with age but without statistical significance.

subjects in their 30s was 0.99 ± 0.6, which was only 40% (P < .01) of the activity found in the group of subjects in their 20s. The enzyme activities of the groups of subjects in their 40s, 50s, 60s, and 70s were 50% (P < .05), 24% (P < .01), 39% (P < .01), and 36% (P < .01), respectively, of the enzyme activities of the group in their 20s (Fig 3). It therefore appeared that the FMO activity declined significantly in subjects in their 30s.

The mean of xanthine oxidase activities observed in the group of subjects in their 20s was 1.22 ± 0.5. Xanthine oxidase activity in the subjects in their 20s was not higher than those in older age groups. Instead, xanthine oxidase activities were similar in all age groups (20s to 70s; Fig 4).

DISCUSSION

CYP1A2, FMO, and xanthine oxidase play important roles in the metabolism of many endogenous and foreign chemicals and drugs. In this study, we determined the effect of age and smoking on CYP1A2, FMO, and xanthine oxidase activities in 133 Korean subjects. To our knowledge, it is novel to use caffeine to assess whether these enzymes are modulated by age and smoking habits from the age-matched groups of subjects in their 20s to those in their 70s, not only in Koreans but also in other ethnic groups.

Urinary molar concentration ratios of combined caffeine metabolites (17U + paraxanthine) and caffeine have been used widely to study the effects of environmental (smoking habits,[6] parasitic infection, and nutrition[18]) and genetic (gender and race[5]) factors on CYP1A2 activity. Earlier studies on the effect of gender on CYP1A2 activity by use of the coffee test have shown that the enzyme activity in women was lower than that of men.[5,15,19] However, other studies reported that the enzyme activity was not influenced by gender both in children[3] and in adults.[20,21] The results obtained in this study with age-matched nonsmoking Korean volunteers showed that CYP1A2 activity was not influenced by gender (Table II). This would indicate that the ability for metabolic activation of procarcinogenic heterocyclic amines or polyaromatic hydrocarbons to their reactive carcinogens by CYP1A2 in men and women is the same. Therefore the increased incidence of bladder cancer in men may be more correctly explained by greater occupational exposure to these procarcinogens in men[22,23] than the higher CYP1A2 activity observed in men.[24]

With use of human liver microsomes obtained from fetuses, neonates, infants, and adults, the effect of age on the rates of caffeine *N*-demethylation producing theobromine, paraxanthine, and theophylline has been determined.[25] Rates of paraxanthine and theophylline

formations catalyzed by CYP1A2 were low in fetuses and began to increase rapidly from neonates to infants and reached a high in adults. CYP1A2 activity determined by the ratio of (AFMU + 1U + 1X)/17U, a different version of coffee test, in the prepubertal children (age range, 4 to 11 years) was higher than that of nonsmoking adults.[3] In support of this, in vivo activities of CYP1A2, 2C9, 2D6, and 3A4 have been reported to be low at birth but maximally increased at the young adult stage and decreased in old age.[26] Results obtained with our age-matched nonsmoking Korean volunteers (Fig 2) appear to support this observation and to indicate that the CYP1A2 activity increases to its maximum by young adulthood and decreases after the age of 29.

Smoking is known to be responsible for 80% to 90% of lung cancers observed in industrialized countries.[27] It is well known that the procarcinogens such as heterocyclic amines and polyaromatic hydrocarbons contained in cigarette smoke are oxidized to reactive metabolites, which serve as proximate carcinogens. Furthermore, the procarcinogens contained in cigarette smoke are also known to enhance the CYP1A2 activity that oxidizes the procarcinogens to their reactive metabolites. In previous studies that used the coffee test, CYP1A2 activity has been shown to increase by cigarette smoking[5,17,28,29] and to decrease after cessation of smoking.[30] Results obtained with our Korean population (Fig 1) confirmed the observations of these previous studies.

Among several isoforms of FMO present in humans, FMO3 is the major form present in the liver and plays a significant role in hepatic drug oxidation. The level of FMO3 expressed in human liver microsomes has been reported to be independent of gender[31,32] and not modulated by environmental chemicals.[9] However, in the livers of rats pretreated with 3-methylcholanthrene (a polyaromatic hydrocarbon known to be present in cigarette smoke) the messenger ribonucleic acid levels of FMO1 (major form in rat liver) and the FMO activities (thiobenzamide *S*-oxidation) were found to be increased by 3.9- and 2.9-fold, respectively.[33] This indicated the possibility that the FMO activity may be increased in smokers. In support of the earlier observation that indicated that the level of FMO3 is not influenced by gender,[31,32] the results obtained in this age and gender-matched nonsmoking Korean population (Table II) showed that the FMO activity was not influenced by gender. However, in support of our previous in vitro study with rat liver,[33] which suggested that the FMO activity may be increased in smokers, the in vivo coffee test results in this study showed that FMO activity is increased in smokers (Fig 1). As observed by Tanaka[26] for several CYP isozymes and as is the case of CYP1A2 activity, the FMO activity in our age-matched nonsmoking Korean population showed that it was significantly higher in the young adults (age range, 20 to 29 years) but lower in older adults (age range, 30 to 70 years; Fig 3). Therefore although the in vivo FMO activity is not influenced by gender, it is increased in smokers and undergoes age-related changes, showing higher activity in younger adults but declining with old age.

The coffee test has also been used to study the effects of gender and smoking on xanthine oxidase activity but with controversial results.[5,19,21] Xanthine oxidase activity has been shown to increase in arthritis, asthma, and hypoxia-reperfusion injury, diseases that occur more frequently in old age.[34] Increased xanthine oxidase activity that causes increased production of reactive oxygen species in inflammatory airway diseases, such as asthma and long-term smoke-derived obstruction, may cause tissue damage.[35] It was therefore of interest to determine whether the in vivo xanthine oxidase activity is altered in smokers and the elderly. Results of the coffee test to determine xanthine oxidase activity in our age- and gender-matched nonsmoking healthy Korean volunteers indicates that the enzyme activity is the same between men and women (Table II). Xanthine oxidase activities were similar in all age groups (subjects in their 20s to 70s; Fig 4). Surprisingly, the enzyme activity of smokers and nonsmokers was the same (Fig 1, *C*). Because all of our volunteers lived in a cosmopolitan urban city, we had expected that accumulated environmental pollutants, particularly in older smokers, would increase the xanthine oxidase activity.

In conclusion, CYP1A2, FMO, and xanthine oxidase activities were assessed with use of urinary caffeine metabolite ratios in 133 age- (age range, 21 to 78 years) and gender-matched healthy Korean volunteers with and without smoking habits. CYP1A2, FMO, and xanthine oxidase activities showed marked individual variations, but there were no gender-related differences for the activities of these enzymes. CYP1A2 and FMO activities were higher in the younger adults and declined in older subjects. However, the xanthine oxidase activities were similar in all age groups (subjects in their 20s to 70s). CYP1A2 and FMO activities were elevated in smokers, but xanthine oxidase activity was not. The results of this study therefore suggest that dosages may need to be adjusted according to the ages and smoking habits of patients when drugs that are metabolized by CYP1A2 and FMO are prescribed.

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