Urinary Excretion of Unmetabolized and Phase II Conjugates of 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline in Humans: Relationship to Cytochrome P4501A2 and N-Acetyltransferase Activity

W. G. Stillwell, La Creis R. Kidd, John S. Wishnok, Steven R. Tannenbaum, and Rashmi Sinha

Division of Toxicology [W. G. S., L. R. K., J. S. W., S. R. T.] and Department of Chemistry [S. R. S.], Massachusetts Institute of Technology, Cambridge, Massachusetts 02139-4307, and Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, Maryland 20892 [R. S.]

ABSTRACT

Cooking meat, fish, or poultry at high temperature gives rise to heterocyclic aromatic amines (HAAs), which may be metabolically activated to mutagenic or carcinogenic intermediates. The enzymes cytochrome P4501A2 (CYP1A2) and N-acetyltransferase (NAT2) are principally implicated in such biotransformations. We have determined the relationship between the activity of these two enzymes and the urinary excretion of unmetabolized and Phase II conjugates of the two HAAs MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) in individuals fed a uniform diet containing high-temperature cooked meat. The subjects in the study ate meat containing known amounts of MeIQx and PhIP, and urine collections were made 0-12 and 12-24 h after a meal. MeIQx and PhIP were measured in urine after acid treatment that quantitatively hydrolyzes the Phase II conjugates to the respective parent amine. The extracts containing the HAAs were purified by immunoaffinity chromatography and analyzed by liquid chromatography using electrospray ionization-tandem mass spectrometry. The MeIQx content in the 0-12 h urine increased after acid hydrolysis by a factor of ~3-21-fold. After acid treatment, the total amount of MeIQx (unmetabolized plus the N-glucuronide and sulfamate metabolites) excreted in the 0-12 h urine was 10.5 ± 3.5% (mean ± SD) of the dose, whereas the total amount of PhIP (unmetabolized plus acetylatable conjugates) in the 0-12 h period was 4.3 ± 1.7% (mean ± SD) of the dose. Linear regression analysis of the amounts of MeIQx and PhIP excreted in the 0-12 h period expressed as a percentage of the ingested dose, for all subjects, gave a low but significant correlation (r = 0.37, P = 0.005). Linear regression analyses showed that lower total MeIQx (unmetabolized plus the N-glucuronide and sulfamate metabolites) in urine was associated with higher CYP1A2 activity, whereas total PhIP (unmetabolized plus conjugated) in urine showed no association to CYP1A2 activity. These results indicate that IA in humans, MeIQx metabolism and disposition are more strongly influenced by CYP1A2 activity than those of PhIP. Linear regression analysis found no association between NAT2 activity and the levels (unmetabolized plus acetylatable conjugates) of MeIQx or PhIP excreted in urine.

INTRODUCTION

The HAAs3 MeIQx and PhIP illustrated in Fig. 1 are two of the principal HAAs that are present in cooked meats (1-3). MeIQx is responsible for a major portion of the mutagenic activity found in cooked beef (4), whereas PhIP exhibits moderate mutagenic activity but is often present in higher amounts in cooked meats compared to the other HAAs (5, 6). MeIQx and PhIP, like other HAAs, require metabolic activation via CYP oxidation to convert them to reactive species with genotoxic activity. The CYP oxidation of HAAs is catalyzed primarily by hepatic CYP1A2 (7-9). Interindividual activity of CYP1A2 shows variability, which is due in part to genetic polymorphisms and environmental factors (10). Dietary factors, including consumption of high-temperature cooked meats, were found to induce CYP1A2 activity in humans (11). The activity of NAT2, an enzyme involved in both the N-acetylation of aromatic amines and the O-acetylation of N-hydroxylamines, is regulated by genetic phenotype and is not modified by environmental factors (12). N-Acetylation of aromatic amines represents a competing pathway for arylamine N-oxidation, whereas the O-acetylation of N-hydroxylamines is regarded as an activation process leading to more reactive intermediates.

The mutagenic fate of MeIQx and PhIP in human subjects and the factors affecting it have been investigated in several studies. Murray et al. (13) found that the excretion of unmetabolized MeIQx in urine ranged between 1.8 and 4.9% of the oral dose. Subsequently, we reported evidence for the elimination of acid-labile conjugates of MeIQx, i.e., the N-glucuronide and N-sulfamate derivatives (14). In the case of PhIP, Lynch et al. (15) reported that the excretion of unchanged PhIP in urine ranged between 0.6 and 2.3% of the oral dose. In an assessment of the intra- and interindividual variability in systemic exposure of MeIQx and PhIP in humans, they found that the urinary excretion of unmetabolized MeIQx and PhIP (expressed as the percentage of the ingested dose) remained relatively constant for an individual, but that intersubject variation was greater. In a later work, Boobis et al. (16) showed that the excretion of unchanged MeIQx and PhIP increased 14- and 4-fold, respectively, after human subjects were treated with furafylline, an inhibitor of CYP1A2. In a dietary study conducted with subjects consuming a controlled meat diet, Sinha et al. (17) found an inverse relationship between individuals with high CYP1A2 activity and excretion of urinary unconjugated MeIQx, whereas NAT2 activity showed no correlation with MeIQx excretion. These data indicate that in humans, CYP1A2 activity has an effect on MeIQx metabolism.

At present, no information is available on the contribution of Phase II conjugation to the metabolism of PhIP in humans nor has the relationship of CYP1A2 or NAT2 activity to the disposition of PhIP been described. The N3-glucuronide metabolite of PhIP was identified as the major detoxification product in studies with human liver microsomes in vitro, and preliminary findings also indicated that this metabolite was excreted in human urine (18). Other studies with human liver microsomes have shown that PhIP is readily converted to the genotoxic product 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine (19).

Recent advances in methodology in human metabolic phenotyping as well as the development of techniques for the analysis of...
arylaminos in biological matrices have allowed associations to be made on enzyme polymorphisms, metabolic pathways, and exposure risks in humans (20). The excretion of urinary metabolites of MeIQx and PhIP could be used as an index of individual exposure to and metabolism of these compounds. In this investigation, we quantify the extent of Phase II conjugation reactions in the disposition and elimination of PhIP and MeIQx in humans consuming high-temperature cooked meat in a controlled dietary study using newly developed immunoaffinity purification and ESI-LC-MS/MS procedures. We also determined the relationship between the urinary excretion of unchanged plus metabolized MeIQx and PhIP within the individuals in the study. Moreover, we examined the role of CYP1A2 and NAT2 activity on the excretion levels of unmetabolized plus conjugated MeIQx and PhIP in urine.

**MATERIALS AND METHODS**

**Chemicals.** PhIP, MeIQx, PhIP-d3-methyl, and MeIQx-d3-methyl were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Isotopic purity of the deuterated standards was determined by LC-MS to be 99.3 and 98.7%, respectively. Stock solutions of deuterated PhIP and MeIQx were prepared in methanol and quantified with a Hewlett-Packard 8452A spectrophotometer (Hewlett-Packard Co., Palo Alto, CA) using extinction coefficient values of 18,133 M⁻¹cm⁻¹ for PhIP at 316 nm (21) and 41,000 M⁻¹cm⁻¹ for MeIQx at 274 nm (22). Stock standard solutions were prepared daily when needed and stored for usage at a later date. The sulfamate and N2-glucuronide metabolites (2,14C)MeIQx were kindly provided by Dr. R. J. Turesky (Nestlé Research Center, Lausanne, Switzerland; Ref. 23). Omnisorb grade solvents obtained from EM Science (Gibbstown, NJ) and high-purity water (double-distilled) were used. Monoclonal antibodies raised against MeIQx and PhIP were used in the preparation of immunoaffinity columns (24). The antibodies were immobilized on CNBr-activated Sepharose 4B (Sigma Chemical Co., St. Louis, MO) at a concentration of 2—5 mg of protein/ml of gel. Blocked gel for precolumns was prepared by treatment of CNBr-activated Sepharose 4b with 0.2 M Tris-HCl (pH 8.0).

**Study Design.** The subjects participating in the study and the protocol followed in the experimental design have been described in detail in a previous report (11). Briefly, the 66 subjects (33 males and 33 females) were recruited from the Beltsville, MD area. The enrollment criteria included being in good health, being a nonsmoker for at least 6 months, taking no medication other than an occasional analgesic, not consuming any atypical diet (including vegetarian), and being able to consume caffeine.

**Determination of CYP1A2 and NAT2 Phenotype.** The subjects were phenotyped for CYP1A2 and NAT2 function by measurement of the urinary caffeine metabolites by the procedures described in detail elsewhere (10). In brief, caffeine and four of its metabolites were quantified by computerized high-pressure liquid chromatography with photo-diode array detection and spectral validation. NAT2 was determined using the molar urinary ratio of 1,7-dimethylxanthine to l-methylxanthine. CYP1A2 was calculated using the molar urinary ratio of 1,7-dimethylinxanthine plus 1,7-dimethyluracil to 1,3,7-trimethylxanthine.

**Meat Preparation and Controlled Dietary Period.** The meat preparation and dietary protocol have been described in detail elsewhere (11). In brief, the subjects ingested a controlled diet containing minced beef cooked at low temperature, with nondetectable amounts of MeIQx and PhIP for 7 days to minimize the exposure to the HAA As of all individuals. On the morning of day 8, the subjects were phenotyped for CYP1A2 and NAT2 activities. That evening, the subjects ate a meal containing minced beef cooked at high temperature. The lean ground beef was made into quarter-pound patties and pan-fried on a griddle at 250°C for 11 min per side. The subjects consumed meals containing differing amounts of meat based on their body weight (3.1—4.4 g meat/kg body weight). The well-done cooked meat in this phase of the study contained 9 ng/g of MeIQx and 32.8 ng/g of PhIP (11). The subjects collected all urine produced in the 0—12- and 12—24-h postmeal period. The urine samples were frozen and stored at −30°C until analysis. In a separate study, the unconjugated MeIQx in the urine was measured by the GC-MS method described previously (13, 15). The results in this analysis were reported earlier (17).

**Hydrolysis of MeIQx and PhIP Conjugates and Urine Samples.** One of the objectives in the present study was to determine the extent of the excretion of exocyclic amine (MeIQx) and ring-nitrogen (PhIP) conjugation products in human urine. Procedures were developed that used acidic hydrolysis of urine in which the metabolites are quantitatively cleaved to liberate the parent HAA. In earlier studies, it was shown that the amine conjugates of MeIQx (the sulfamate and N2-glucuronide metabolites) are labile to 1 N HCl at 70°C (14). In the present work, optimal conditions for the hydrolysis of the urine samples were established by spiking control urine (10 ml) with radiolabeled [14C]MeIQx-N2-glucuronide and sulfamate standards. The solutions were made 1 N by the addition of 2 ml of 6 N HCl and incubated at 70°C for various time periods. The samples were then neutralized, and the pH was adjusted to 9.5 by the addition of sodium carbonate, extracted with ethyl acetate (2 × 2 volume each), transferred to flasks, and dried under vacuum. The recovery of MeIQx was measured by liquid scintillation counting. With each set of experiments, control samples were analyzed in which no acid treatment was performed. Radiolabeled PhIP-N3-glucuronide was not available for establishing optimal hydrolysis conditions; however, a comparison of the recovery of PhIP was made on urine samples that were either: (a) acid hydrolyzed at 70°C for 4 h; or (b) enzymatically hydrolyzed (18) overnight at 37°C with 1000 units/ml of bacterial β-glucuronidase (EC 3.2.1.31), type X-A from Escherichia coli (Sigma Chemical Co.).

Based on the above experiments, urine samples in the study were hydrolyzed in 1 N HCl for 4 h at 70°C and extracted using the following conditions: 10 ml urine samples were transferred to 50-ml glass-stoppered glass tubes, spiked with a known amount of the internal standards (typically 5 ng each of PhIP-d3 and MeIQx-d6), acidified by the addition of 2 ml of 6 N HCl, and tightly stopped. After heating for 4 h at 70°C, the samples were cooled and neutralized by the addition of 2 ml of 6 N NaOH. Following the addition of 0.5 g of Na2CO3, the samples were extracted twice with two volumes of ethyl acetate. The organic phase was placed in a −16°C freezer for 30 min and then decanted into a glass centrifuge tube containing 1.5 ml of 0.1 N HCl. After extraction, the acid was transferred to a vial, and a second extraction of the organic phase was performed with 1.0 ml of 0.1 N HCl. The acidic extracts were combined and placed into vials and dried by vacuum centrifugal evaporation (Savant Instruments, Inc., Farmingdale, NY).

**Immunoaffinity Purification.** The dried samples were redissolved in 3 ml of PBS [10 mM phosphate buffer (pH 7.4), 0.14 M NaCl], and the pH was adjusted to 8.0 by the addition of 0.2—0.4 ml of 0.1 M NaOH. The solutions...
were passed through precolumns (1 ml of blocked gel) directly onto PhIP and MelQx monoclonal antibody columns (1.5 ml) placed in series. The first eluant solution was collected and passed through the precolumn and antibody columns a second time. The precolumn and antibody columns were then washed with 15 ml of PBS, the precolumns were removed, and an additional 15 ml of PBS solution was applied individually to each PhIP and MelQx antibody column. The respective antibody columns were washed with an additional 20 ml of distilled water. PhIP and MelQx were individually eluted from the antibody columns with 3 ml of 1 N acetic acid. The acidic fractions were collected into separate vials and dried under vacuum. The dried samples were subsequently redissolved in 0.3 ml of 0.5 M Na₂CO₃ and extracted with ethyl acetate (2 × 0.5 ml). The organic layer was dried under a stream of nitrogen and subsequently transferred with a small volume of ethyl acetate (2 × 90 μl) to microvolume glass inserts positioned in small screw-cap vials (DP-Target; Hewlett-Packard Co.). The samples were dried under a gentle flow of nitrogen and stored in a −16°C freezer until analysis by LC-ESI-MS/MS.

For further reuse, the monoclonal antibody columns were washed with additional 1 N acetic acid (20 ml), followed with distilled water (20 ml), then with sodium bicarbonate buffer (0.1 M, pH 8.3) containing NaCl (0.5 M), and finally with PBS solution (20 ml containing 2 mM sodium azide). The columns were then stored in PBS at 4°C for later use.

**LC-MS/MS Analysis.** LC-MS/MS analyses of PhIP and MelQx in the purified extracts were carried out using an on-line high-pressure liquid chromatography (Hewlett-Packard 1090) coupled to a Finnigan TSQ 7000 triple-stage quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA) equipped with an electrospray ionization source. A Vydac narrow-bore C₁₈ reverse-phase column (150 × 2.1 mm, 5-μm particle size) was used at a flow rate of 200 μl/min. The mobile phase consisted of methanol:water (50:50) containing 0.1% formic acid. The HAAs were analyzed in positive ion mode by the technique of selected reaction monitoring in which the triple-stage quadrupole mass spectrometer was operated under collision-induced dissociation conditions to monitor the transition of a precursor ion to its corresponding product ion. For PhIP, the transition of the protonated molecular ion [M + H]⁺ → m/z 210 (loss of a methyl or d₃-methyl group) was monitored, whereas for MelQx, the analogous transition of the protonated molecular [M + H]⁺ → m/z 199 was monitored. The instrumental parameters of the triple-stage quadrupole mass spectrometer were optimized for the analysis of the respective HAA by adjusting the capillary voltage and tube lens voltage for maximum sensitivity. For the analysis of PhIP, the collision voltage was set at −32 V, and a pressure of 3.2 mtorr argon was maintained in the collision cell. For the analysis of MelQx, the collision voltage was set at −29 V, and a pressure of 2.9 mtorr argon was maintained in the collision cell. The heated capillary was set at 240°C, while the nitrogen sheath gas was held at 80 psi, and the auxiliary nitrogen gas set at 18 on the dial. Alternate measurement of the deuterated internal standards and the unlabeled HAA parent ions to the same product ions was performed by the Finnigan data system. The amounts of the HAAs in the samples were determined by the ratios of the integrated areas of the ion chromatograms of the respective product ions. Deuterated standards (d₃) were analyzed under the same ESI-MS/MS conditions to correct for background contribution of the amount of the unlabeled (d₀) MelQx and PhIP present in the
Figure 3. ESI-LC-MS/MS analysis of PhIP in human urine. The selected reaction-ion traces are of PhIP (upper trace) and the δ7-internal standard (lower trace). The concentration of PhIP in the sample was 460 pg/ml.

Table 1 Characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
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<td>27-62</td>
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<tr>
<td>Weight (kg)</td>
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<td>42.2-102.5</td>
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<td>BMI (kg/m²)</td>
<td>23.9</td>
<td>17.0-32.1</td>
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<td>Amount of meat eaten (g)</td>
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<td>180-318</td>
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<tr>
<td>NAT2 activity</td>
<td>0.6</td>
<td>0.19-3.7</td>
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<td>CYP1A2 activity</td>
<td>8.8</td>
<td>2.1-28</td>
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</table>

Table 2 Urinary excretion of MelQx and PhIP

<table>
<thead>
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<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeIQx in urine (ng)</td>
<td>230 ± 88</td>
<td>221</td>
<td>72-644</td>
</tr>
<tr>
<td>PhIP in urine (ng)</td>
<td>351 ± 160</td>
<td>322</td>
<td>116-1012</td>
</tr>
<tr>
<td>MeIQx % of dose 0-12 h</td>
<td>10.5 ± 3.5</td>
<td>10.4</td>
<td>3.2-22.7</td>
</tr>
<tr>
<td>PhIP % of dose 0-12 h</td>
<td>4.3 ± 1.7</td>
<td>4.0</td>
<td>1.9-9.8</td>
</tr>
<tr>
<td>PhIP % of dose 12-24 h</td>
<td>0.9 ± 0.4</td>
<td>0.9</td>
<td>0.5-1.9</td>
</tr>
</tbody>
</table>

* Total of unmetabolized and acid-labile metabolites.
RESULTS

Hydrolysis Conditions. The recoveries of [14C]MeIQx were determined after urine samples were spiked with the radiolabeled MeIQx-$N^2$-glucuronide or sulfamate standards and hydrolyzed in 1 N HCl at 70°C for varying times. Urine samples that were not hydrolyzed had recoveries that were less than 0.8% of the total amount added. With the addition of acid and an incubation time of 4 or 6 h, the recovery of MeIQx from the sulfamate metabolite was 72% of the total amount, whereas for the $N^2$-glucuronide conjugate, the recovery was approximately 85% of the total. In the case of PhIP, a comparison of recoveries from urine samples that were either acid hydrolyzed or enzymatically hydrolyzed showed similar results when analyzed for PhIP content, thus indicating that acid hydrolysis in 1 N HCL at 70°C for 4 h is sufficient for the hydrolysis of the expected $N^3$-glucuronide metabolite in urine.

LC-MS/MS Characteristics. The LC-ESI mass spectra of MeIQx and PhIP were recorded in the positive ion mode. The two compounds showed abundant protonated molecular ions [M + H]$^+$ at $m/z$ 214 and 225, respectively. Under collision-induced dissociation conditions, both HAAs readily lose a methyl group to yield the corresponding [MH-15]$^+$ fragment ions in high abundance; little other fragmentation was observed. Selective ion-reaction monitoring was used to analyze the aforementioned precursor and product ions of the HAAs and their respective stable-isotope labeled internal standards. The transition of the protonated molecular ion and internal standard at $m/z$ 225 and 228 $\rightarrow m/z$ 210 was monitored for the analysis of PhIP, whereas for MeIQx, the analogous transition of $m/z$ 214 and 217 $\rightarrow m/z$ 199 was monitored. The precursor to product ion reactions involved in selective reaction monitoring provides great specificity of detection, whereas the use of immunoaffinity column purification produces samples that are clean and amenable for direct analysis by LC-MS/MS. Good detection limits in the low picogram range were obtained for the standard compounds injected on column with no detectable background interference. The calibration curves for both MeIQx and PhIP showed good linearity over the range of 0.5–15 ng/sample.

Urine Analysis. The results of MeIQx and PhIP analysis in human urine using selected ion-reaction monitoring showed detection limits in the low (5–10) picogram/ml range for both MeIQx and PhIP. At these levels, the signal:noise ratio was greater than 5 for each HAA. Overall recovery of PhIP and MeIQx from urine through the entire procedure is 25% or greater based on the analysis of unextracted deuterated internal standards. The precision of the urinary assay was determined by replicate analyses of MeIQx and PhIP from aliquots of a pooled urine collection. The quality control samples were assayed as described above and analyzed over a 4-month period. The percent coefficient of variation for the urinary analysis of MeIQx was 16, whereas the percent coefficient of variation for PhIP was 31. Illustrated in Figs. 2 and 3 are typical ion traces obtained from selected reaction monitoring of the precursor to product ions of MeIQx and PhIP and their respective deuterated internal standards isolated from human urine. These results characterize the high degree of selectivity and the lack of interference from matrix or endogenous components in these analyses.

Subject Characteristics and Urinary Excretion of MeIQx and PhIP. Table 1 summarizes the subject characteristics, the quantity of meat eaten, and other variables. The content of MeIQx and PhIP in the high temperature-cooked meat was 9.0 ng/g and 32.8 ng/g, respectively, and the quantity of meat eaten for each individual was 3.1–4.4
and estimated SE, the level of significance (P value) for the meat

tion (P = 0.55). The intercept (p0), model R2, slope of the fitted line

No correlation was found between CYP1A2 activity and PhIP excre

cipitation in urine and only minor quantities (0.2—1.3% of the dose)

tural diet under controlled dietary conditions. The data reported here provide evidence that in humans, urinary Phase II detoxification metabolites in the elimination of unoxidized MeIQx are comparatively higher than are those of unoxi-
dized PhIP. The participants in this study excreted an average of

The total amount of urinary PhIP measured as unchanged plus

acid-labile conjugate(s) in the 0—12-h urine accounted for an average of 4.3% of the dose in the 12—24-h period following the consumption of a uniform diet of well-done cooked beef (17). The median and range of these enzyme values are shown in Table 1. Tests for the association of CYP1A2 activity on total urinary MeIQx and PhIP excretion were conducted. Fig. 5a illustrates the effect of CYP1A2 on total (unmetabolized plus conjugated) urinary MeIQx excretion normalized to the median of meat consumed. The slope of the fitted line shows the average change in the log10 of total MeIQx per unit change in log10 CYP1A2 activity. The subjects with higher CYP1A2 activity have less total MeIQx in the urine than the subjects with lower enzyme activity. CYP1A2 activity showed a notable (P = 0.02) influence on total MeIQx excreted in urine. Tests for the association of CYP1A2 activity with other individual characteristics, such as age, gender, weight, and body mass index and tests for the importance of these features as main effects were all negative. The relationship between CYP1A2 activity and total (unmetabolized plus conjugated) urinary PhIP, adjusted for the amount of meat intake, is depicted in Fig. 5b. No correlation was found between CYP1A2 activity and PhIP excretion (P = 0.55). The intercept (β0), model R2, slope of the fitted line and estimated SE, the level of significance (P value) for the meat intake (β1), and enzyme activity (β2) for both MeIQx and PhIP are given in Table 3.

Table 3 Variables examined in relationship to total (unmetabolized plus conjugated, log10 transformed) urinary MeIQx and PhIP

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>β-coefficient (SE)</th>
<th>P</th>
<th>Intercept</th>
<th>R^2</th>
</tr>
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<tbody>
<tr>
<td>MeIQx</td>
<td>Multiple linear regression</td>
<td>0.002 (0.0006)</td>
<td>0.003</td>
<td>2.07</td>
</tr>
<tr>
<td>Amount of meat consumed</td>
<td></td>
<td>-0.20 (0.08)</td>
<td>0.06</td>
<td>0.02</td>
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<tr>
<td>Log10 CYP1A2</td>
<td></td>
<td>-0.06 (0.1)</td>
<td>0.001</td>
<td>1.89</td>
</tr>
<tr>
<td>PhIP</td>
<td>Multiple linear regression</td>
<td>0.003 (0.0006)</td>
<td>0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Amount of meat consumed</td>
<td></td>
<td>-0.69 (0.08)</td>
<td>0.06</td>
<td>0.02</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we determined the contribution of Phase II conjugation reactions to the metabolism and disposition of MeIQx and PhIP in individuals consuming a uniform diet under controlled dietary conditions. The data reported here provide evidence that in humans, urinary Phase II detoxification metabolites in the elimination of unoxidized MeIQx are comparatively higher than are those of unoxidized PhIP. The participants in this study excreted an average of 9-fold more MeIQx (expressed as percentage of the dose) in the form of acid-labile conjugates in urine than unmetabolized MeIQx. These results are in agreement with an earlier study showing that the N2-glucuronide and sulfamate metabolites of MeIQx are important elimination products in humans (14). In the case of PhIP, a previous investigation found that the urinary excretion of unchanged PhIP accounted for an average of 1.1% of the ingested dose (15). In the present study, the excretion of PhIP [unmetabolized plus acid-labile conjugate(s)] in the 0—12-h urine accounted for an average of 4.3% of the ingested dose, whereas another 0.9% of the dose was excreted in the 12—24-h urine. Thus, approximately 4-fold more PhIP in the form of acid-labile metabolites than unmetabolized PhIP is excreted in the combined 0—24-h urine of the subjects. Several variables may influence the comparatively lower amount of PhIP excreted in urine compared to MeIQx. These are differential rates of Phase II conjugation reactions that form the polar metabolites of PhIP for excretion and/or a preferential excretion of these metabolites of PhIP in feces.

A comparison of the amounts of MeIQx and PhIP in urine expressed as the percentage of the dose ingested, for all subjects, following consumption of a uniform diet of well-done cooked beef showed a low correlation, indicating that metabolic variation may be important in the disposition and elimination of these HAAs. Differences were evident in the relationship between CYP1A2 activity and total (unmetabolized plus conjugated) urinary excretion of MeIQx and PhIP. The results reported here are in accordance with the earlier study and show that higher CYP1A2 activity is correlated with lower MeIQx excretion represented in terms of either unmetabolized MeIQx (17) or unmetabolized MeIQx plus the N2-glucuronide and sulfamate metabolites. N2-Deamination and sulfamation of MeIQx represent competing pathways for arylamine N-oxidation; thus, an inverse relationship would be expected for the comparison of total MeIQx (unmetabolized plus amine conjugated) excreted versus CYP1A2 activity. The urinary excretion of total PhIP (unmetabolized plus conjugated) did not show an inverse relationship to the enzyme activity of CYP1A2. These results suggest that the metabolism of MeIQx in humans is more strongly influenced by CYP1A2 activity than is that of PhIP. These findings are in agreement with the previous
in vivo investigation showing that the excretion of unchanged MeIQx in humans was increased by a greater degree after treatment with furafylline (an inhibitor of CYP1A2) than was PhIP (16). In contrast, experimental studies (9) with human liver microsomes have shown that the rate of N-oxidation of PhIP by CYP1A2 is comparatively high with respect to other HAAs. The lack of correlation between CYP1A2 activity and urinary PhIP excretion may involve other factors, such as detoxification reactions of the N-acetoxy derivative of PhIP with glutathione. It was reported (25) that certain human hepatic glutathione S-transferases are active in detoxifying N-acetoxy-PhIP via a redox reaction to form PhIP and that this activity did not occur with the N-acetoxy derivatives of MeIQx or the related food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline. Other human cytochrome P-450s, including cytochrome P-4501A1, may also be involved in the metabolism of PhIP (26, 27). Recent evidence has been reported that human cytochrome P-4501B1 expressed in yeast is involved in the metabolic activation of MeIQx and to a lesser extent PhIP (28).

Metabolism studies in nonhuman primates have demonstrated that PhIP is metabolized to several polar metabolites including the N3-glucuronide conjugate of N2-hydroxy-PhIP (29). This metabolite was detected in small amounts in the urine, bile, and serum of treated animals and is believed to act as a transport form for the N-hydroxy-l-arginine to extrahepatic tissues. Our studies showed that in humans, PhIP undergoes conjugation reactions to form an acid-labile metabolite(s) which is eliminated in urine. It remains to be determined whether the N2-hydroxy-N3-glucuronide of PhIP is also a metabolite product and as such excreted in human urine.

No correlation between the enzyme activity of NAT2 and MeIQx or PhIP excretion was found. These findings are consistent with the results of an investigation showing that MeIQx and PhIP are not N-acetylated by human liver cytosol (9). Other investigations demonstrated that MeIQx was not N-acetylated by human NAT2 expressed in COS-1 cells, whereas PhIP showed a markedly low rate of N-acetylation (30). Thus, NAT2 activity would not be expected to yield a direct comparison of the extent of human exposure of these compounds. Analysis of unmetabolized MeIQx in urine may provide a better estimate or biomarker for monitoring the degree of exposure to this HAA in human studies (15). The excretion of unmetabolized PhIP may be too low to be useful as a biomarker.

REFERENCES


Urinary Excretion of Unmetabolized and Phase II Conjugates of 2-Amino-1-methyl-6-phenylimidazo[4,5-\textit{b}]pyridine and 2-Amino-3,8-dimethylimidazo[4,5-\textit{f}]quinoxaline in Humans: Relationship to Cytochrome P4501A2 and \textit{N}-Acetyltransferase Activity
