Metabolism of the Food-borne Carcinogens 2-Amino-3-methylimidazo[4,5-f]quinoline and 2-Amino-3,8-dimethylimidazo[4,5-f]-quinoxaline in the Rat As a Model for Human Biomonitoring

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Introduction

Methods have been developed to rapidly quantitate carcinogenic heterocyclic amines formed in cooked foods such as meat and fish (1–4). However, measurement of these contaminants in food only provides a crude estimate of exposure and does not account for absorption and metabolism of these procarcinogens to biologically active species or to detoxified products. Many of these mutagens contain an aminimidazole group as a common structural feature. 2-Amino-3-methylimidazo[4,5-f] quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) are two important representatives of this class of contaminants. We have used the rat as an animal model to elucidate routes of metabolism and disposition of these two compounds. Particular emphasis has been placed on the analysis of acid-labile sulfonamide protein adducts derived from reactive N-hydroxy metabolites and on the analysis of nontoxic metabolites excreted in urine for developing noninvasive methods of human biomonitoring. We have observed that human liver tissue also transforms heterocyclic amines to reactive mutagens as well as...
to detoxified products. Our recent data on the quantification of heterocyclic amines in cooked foods, the use of the rodent model for developing methods of human biomonitoring, and preliminary data from human studies are presented in this article.

Methods

Heterocyclic amines were isolated from heated meat products by adsorption to XAD-2 resin followed by blue cotton treatment or by immunoffinity chromatography. Quantification was done by HPLC with UV or mass spectrometry detection (2,3). Analysis by solid-phase tandem extraction gave similar values, but enabled the measurement of other heterocyclic amines, including 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-9H-pyrido[2,3-b]indole (2-AcC), which do not cross-react with the antibodies (4).

Male Sprague-Dawley rats were used for protein adduct and metabolism studies (5,6). In brief, hemoglobin was purified by lysis of erythrocytes, followed by gel filtration chromatography. Serum albumin was purified by affinity chromatography using Cibroan Blue conjugated to sepharose. Acid-labile sulfonamide protein adducts were quantitated by HPLC. Metabolites were isolated from biological fluids and reference standards were prepared biosynthetically using rat hepatocyte suspensions or through chemical syntheses (5,7).

Analysis of MeIQx excreted in urine of humans following consumption of fried beef was done by the method of Murray et al. (8) using negative-ion chemical ionization GC-MS except that an immunoffinity purification step was included following the acid/base partitioning step (3). Three subjects ingested 1 lb equivalent cooked beef prepared under typical household cooking practices and then collected urine for 24 hr. Trideuterio-labeled (N-Cd3)-MeIQx was used as an internal standard. MeIQx and d3-MeIQx were monitored at the ions m/z 438 and m/z 441 [corresponding to the M-227 ions of the di(3,5-bistri- fluoromethylbenzyl)derivatives]. Human hepatic microsomes and cytosols were used to measure the apparent rates of N-oxidation and N-acetylation of heterocyclic amines (9). S-9 fortified with adenosine 3'-phosphate-5'-phosphosulfate (PAPS) was used to examine sulfamate formation.

Results

Heterocyclic amines can be detected at the low parts per billion levels in meat and fish prepared under typical household cooking practices. The production of these genotoxins in foods varies greatly and depends on the meat, temperature, and manner of preparation (Table 1).

The major metabolites of IQ and MeIQx that have been identified in urine, bile, and feces of rodents are displayed in Figure 1 (5,10-12). All these metabolites are detoxification products with the exception of the N-glucuronide conjugate of N-hydroxy-MeIQx, which is genotoxic in the presence of β-glucuronidase (7).

We examined the metabolism of MeIQx at doses ranging from 0.01 to 20 mg/kg in noninduced rats and in rats pretreated with polychlorinated biphenyls (PCB) (5) (Fig. 2). At high dose exposure to MeIQx (20 mg/kg), the sulfamate and N2-glucuronide were the major metabolites excreted in urine, whereas PCB-pretreated animals excreted greater amounts of conjugates of 5-hydroxy-MeIQx. Cytochrome P-450 induction had no influence on metabolism at the 0.01 mg/kg dose, indicating that under high exposure to MeIQx the cytochrome P-450 is limiting in the noninduced rat and phase II conjugation reactions make a larger contribution to metabolism. Notably, the formation and excretion of the metastable N-glucuronide conjugate of N-hydroxy-MeIQx was relatively more important at low-dose exposure.

The blood protein binding of IQ was examined at doses from 2 to 150 μmole/kg (6). Among many proteins modified, hemoglobin and albumin were modified in a dose-dependent fashion. Albumin bound three to five times more IQ than hemoglobin per mole of protein. The amount of IQ bound to the total albumin pool ranged from 1.4 to 4.3 × 10^-2 % of the dose (Fig. 3). Analysis of the enzymatically digested peptide fragments revealed many adducts. One adduct was identified as a tripeptide containing an N2-cysteine sulfanamide-IQ linkage and accounted for as much as 10% of the IQ bound to serum albumin (Fig. 4). A chemically identical adduct was formed in vitro when N-hydroxy-IQ was incubated with serum albumin. The adduct was labile to acid with quantitative recovery of the parent amine.

| Table 1. Heterocyclic aromatic amines in cooked meats and fish.* |
|----------------|----------------|----------------|----------------|----------------|
| Meats          | 3 components of cooking method | IQ | MeIQx | 4,8-DiMeIQx | PhIP | 2-AcC |
| Beef steak     | Grilled, 190°C, 3 min side       | ND | 5.1   | 1.3          | 23.5 | 3.2   |
|                | Grilled, 190°C, 7 min side       | ND | 8.3   | 2.0          | 48.5 | 8.9   |
| Ground beef    | Pan fried, 250°C, 5 min side     | ND | 0.7   | ND           | NA   | NA    |
|                | Pan fried, 250°C, 10 min side    | ND | 1.1   | <1           | 1.2  | NA    |
| Ground beef    | Pan fried, 250°C, 10 min side    | 0.3 | 4.2   | ND           | NA   | NA    |
| Salmon         | Pan fried, 200°C, 3 min side     | ND | 1.4   | ND           | 1.7  | ND    |
|                | Pan fried, 200°C, 9 min side     | ND | 4.7   | ND           | 14.0 | 8.0   |
| Salmon         | Barbecued, 270°C, 4 min side     | ND | <1    | ND           | 2.0  | 2.8   |
|                | Barbecued, 270°C, 9 min side     | ND | <1    | ND           | 69.0 | 73.0  |
| Bacterial-grade beef extract | I | 49.0 | 56.0 | ND | NA | NA |
|                 | II | 70.0 | 89.0 | 8.1 | NA | NA |
| Food-grade beef extract | I | 6.2  | 30.5 | ND | NA | NA |
|                 | II ND | 69.0 | ND | NA | NA | NA |

Abbreviations: IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; 2-AcC, 2-amino-9H-pyrido[2,3-b]indole; ND, not detected (less than 0.3 ppb); NA, not analyzed.

*From Turesky et al. (2,3), Gross (4), and Gross and Grütter (24).
Adducts bound to hemoglobin were briefly examined for the presence of a sulfinamide adduct. Acid hydrolysis released approximately 30% of the bound IQ. Analysis by HPLC revealed that the product(s) were more polar than IQ and excluded the presence of a sulfinamide linkage with hemoglobin. The chemical structures of the polar products have not been elucidated.

The binding of MelQx to albumin and hemoglobin was several fold lower than that observed for IQ. Hepatic cytochrome P-450 induction with PCB increased the rate of formation of the biologically reactive N-hydroxy metabolite by 20-fold in microsomal assays, but resulted in a 10-fold decrease in blood protein binding in vivo (5). Thus, in contrast to other aromatic amines that are converted to the hydroxylamine and adduct to hemoglobin as a sulfinamide linkage through the 9β cysteine residue in vivo (13), IQ and MelQx apparently do not form this adduct at appreciable levels.

The ability of human liver tissue to metabolize heterocyclic amines was examined. The apparent rates of N-oxidation were compared with that of 4-aminobiphenyl (ABP), which is regarded as the most potent of arylamine carcinogens. The levels of IQ, MelQx, and Glu-P-1 N-oxidation were about half that observed for ABP, while that of PhIP was slightly higher (Table 2). Heterocyclic amines were poor substrates for cytosolic N-acetyltransferases which was in contrast to the arylamine carcinogens ABP or 2-aminofluorene (14). (Table 2). However, human liver S-9 fraction fortified with PAPS was found to readily detoxify IQ and MelQx through sulfamate formation (R. Turesky, unpublished observations).

The urine of three subjects was examined for MelQx before and after consumption of 1 lb of cooked beef. MelQx could not be detected in urine collections before consumption of meat, but the mutagen was detected in all three subjects after the meal (Fig. 5). The amounts of MelQx recovered ranged in values from 6 to 10 to 18 ng in a 24 hr urine collection. These values are similar to those of Murray et al. (8) and indicate that MelQx is absorbed and extensively biotransformed by humans.

**Discussion**

Heterocyclic amines formed in cooked foods at the low part per million level are easily isolated by immunoaffinity chromatography or by solid-phase tandem extraction and then quantitated by HPLC. The class and the amounts of heterocyclic amines produced depend on several parameters including temperature, creatinine content, and meat preparation (15). PhIP, followed by AαC and MelQx, were the most abundant heterocyclic amines found in grilled steak, fried beef, and salmon as well as in barbecued salmon. IQ and MelQx were the predominant mutagens formed in meat extracts. These amounts are comparable to those reported by other investigators who used far more laborious methods of purification (15,16). Based upon these analyses, the daily exposure to each of these amines through the diet may be estimated at approximately 100 ng to 10 μg per day.
The metabolism and blood protein binding of IQ and MeIQx in the rodent were examined to develop strategies for human biomonitoring. Measurement of blood protein adducts has been successfully used to assess human exposure and metabolic activation for several different carcinogens, including 4-aminobiphenyl, aflatoxin, and several polycyclic aromatic hydrocarbons (13, 17). The rodent model has been found to be a good surrogate for blood protein adduct formation by several of these carcinogens including the arylamine ABP, where over 5% of an administered dose is bound to hemoglobin as a sulfinamide linkage. This adduct is the result of a series of reactions between the hemoprotein and the carcinogenic N-hydroxy metabolite. The adduct is stable in vivo, but it can be cleaved in vitro with quantitative regeneration of the parent amine. Thus, measurement of this adduct is an indirect measure of metabolic activation and the biologically effective dose. Relative to ABP, the binding of IQ and MeIQx to hemoglobin was quite low, accounting for approximately 0.01% of the dose, and sulfinamide adducts could not be detected (5, 6).

Induction of hepatic cytochrome P-450 by PCB, which increased rates of formation of the hydroxylamine metabolites, actually resulted in as much as a 10-fold decrease in protein binding (5, 6). Incubation of the microsomal hydroxylamines or the synthetic N-hydroxy derivatives of IQ and MeIQx in vitro with erythrocytes generated methemoglobinemia and sulfinamide adduct formation. Thus, the hydroxylamines can penetrate the erythrocyte and react with the hemoprotein. The absence of such an adduct in vivo suggests that either very low levels of the N-hydroxy metabolites are excreted by the liver into the blood stream or that other routes of biotransformation are of far greater importance than N-hydroxylation. Based on the rodent model, formation of sulfinamide adducts in humans would not be expected at appreciable levels, and human dosimetry may prove difficult. In support of this conclusion, a preliminary study assaying for hemoglobin sulfinamide adducts of MeIQx in hu-
Table 2. Comparative rates of N-oxidation by human liver microsomes and N-acetylation by human liver cytosols.

| Substrate | Rate, nmol/min/mg protein ± SD |
|-----------|--------------------------------|
| ABP       | 5.00 ± 0.32                    |
| MelQx     | 2.98 ± 0.49                    |
| IQ        | 2.30 ± 0.21                    |
| PhIP      | 5.34 ± 0.64                    |
| Glu-P-1   | 2.42 ± 0.14                    |
| N-acetylationa | 0.11 ± 0.09          |
| ABP       | 1.70 ± 0.24                    |
| 2-AF      | 2.35 ± 0.55                    |
| MelQx     | <0.05                          |
| IQ        | <0.05                          |
| PhIP      | <0.05                          |
| Glu-P-1   | <0.05                          |
| PABA      | 0.11 ± 0.09                    |

Abbreviations: ABP, 4-aminobiphenyl; MelQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoline; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; Glu-P-1, 2-amino-6-methylpyrido[1,2-a:3',2'-d]imidazole; 2-AF, 2-aminofluorene; PABA, p-aminobenzoic acid.

*Rates of N-oxidation were determined with hepatic microsomes from the same individual (n = 3, 4). Similar results were obtained with microsomes from two other individuals (9,14).

**These cytosols were selected from rapid acetylator individuals (n = 3) whose status was indicated by the high rates of N-acetylation of 2-AF and ABP (9,14).

Humans showed that the adducts, if present, were below levels of detection using GC-MS (18).

Approximately 0.001–0.004% of an administered dose of IQ bound to serum albumin in the rat as a sulfinamide adduct. In man, with a half-life of 20 days for albumin turnover, the adduct level resulting from chronic exposure is approximately 30 times greater than that produced by a 1-day exposure (13). Assuming an human exposure of 1 μg/day with the same adduct binding efficiency as the rat, the amount of serum albumin modified from chronic exposure in a 70-kg individual containing 130 g of albumin would be approximately 2–8 pg of IQ bound/g of albumin. With this level of modification in humans, methods of enrichment would be required to measure covalent binding of sulfinamide albumin adducts by GC-MS.

We undertook urinary metabolism studies in the rodent with MelQx at doses from 20 mg/kg to 10 μg/kg, a dose that is only several hundred-fold greater than the daily human exposure. MelQx was extensively metabolized at the lowest dose examined with only 0.5–2% of the total dose recovered in urine found as unchanged MelQx. In humans, the amount of MelQx recovered in urine represented only several percent of the ingested dose and indicates that this procarcinogen is also absorbed and extensively metabolized by humans (8). The data of Murray et al. (8), as well as data presented here, demonstrate that.

**Figure 5.** Selected monitoring of 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MelQx) (m/z 438) and its trideutero-labeled derivative (m/z 441) in human urine following consumption of fried meat. The sample contained 11 pg MelQx/mL urine, giving a total excretion of 10 ng in the 24-hr urine collection.
GC-MS analysis is sufficiently sensitive to detect MelQx in urine following consumption of fried meat.

Bioformation of heterocyclic amines by human tissues resembles that of rodents in several instances. Metabolic activation through \(N\)-hydroxylation by hepatic cytochrome P-450 IA2 is comparable for both species (19–21). Both rodent and human liver \(O\)-acyltransferases catalyze binding of the \(N\)-hydroxy metabolites to DNA-bound products, yet the parent amines are poor substrates for hepatic \(N\)-acyltransferases (9,21). The \(N\)-hydroxy metabolites have also been shown to be substrates for rodent and human hepatic glucurononyltransferases (5,7,10,22), and the resulting metastable \(N\)-glucuronide conjugates may be implicated in colorectal carcinogenesis (22). A major route of detoxification of heterocyclic amines in rodents, in particular for IQ and MelQx, is through sulfamate formation. This route of metabolism for aromatic amines is relatively uncommon, owing in part to the liability of the arylsulfamate bond. We have observed that human liver tissue also converts these two amines to their respective sulfamate derivatives (R. Turesky, unpublished observations).

The major oxidative pathways of detoxification of IQ and MelQx found in the rodent (5,10–12), have also been identified in the monkey (23; R. Turesky and E. Snyderwine, unpublished observations). Other important routes of detoxification were through sulfamate and \(N^2\)-glucuronide formation. Thus, there is a strong possibility that humans may also transform these compounds in similar fashions. Investigations are underway for detecting some of these polar metabolites of MelQx that may be present in human urine, in particular, the \(N\)-hydroxy-\(N\)-glucuronide, which is an indirect measurement of metabolic activation. The development of such biomarkers may enable us to better evaluate the health risk of chronic dietary consumption of low amounts of heterocyclic amines.

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REFERENCES

1. Sugimura, T. Successful use of short-term tests for academic purposes: their use in identification of new environmental carcinogens with possible risk for humans. Mutat. Res. 205: 33–39 (1988).
2. Turesky, R. J., Bur, H., Huynh-Ba, T., Aeschbacher, H. U., and Milon, H. Analysis of mutagenic heterocyclic amines in cooked beef products by high performance liquid chromatography in combination with mass spectrometry. Food Chem. Toxicol. 26: 501–509 (1988).
3. Turesky, R. J., Forster, C. M., Aeschbacher, H. U., Würzner, H. P., Skipper, P. L., Trudel, L. J., and Tannenbaum, S. R. Purification of the food-borne carcinogens 2-amino-3-methylimidazo[4,5-f]quinoxaline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in heated meat products by immunoaffinity chromatography. Carcinogenesis 10: 151–156 (1989).
4. Gross, G. A. Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. Carcinogenesis II: 1597–1603 (1990).
5. Turesky, R. J., Markovic, J., Bracco-Hammer, I., and Fay, L. B. The effect of dose and cytochrome P450 induction on the metabolism and disposition of the food-borne carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in the rat. Carcinogenesis 12: 1847–1855 (1991).
6. Turesky, R. J., Skipper, P. L., and Tannenbaum, S. R. Binding of 2-amino-3-methylimidazo[4,5-f]quinoxaline to hemoglobin and albumin in vivo in the rat. Identification of an adduct suitable for dosimetry. Carcinogenesis 8: 1537–1542 (1987).
7. Turesky, R. J., Bracco-Hammer, I., Markovic, J., Richli, U., Kappeler, A. M., and Welti, D. H. The contribution of \(N\)-oxidation to the metabolism of the food-borne carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in rat hepatocytes. Chem. Res. Toxicol. 3: 524–535 (1990).
8. Murray, S., Goodnerham, N. J., and Davies, D. S. Detection and measurement of MelQx in human urine after ingestion of a cooked meat meal. Carcinogenesis 10: 763–765 (1989).
9. Turesky, R. J., Lang, N. P., Butler, M. A., Teitel, C. H., and Kadlubar, F. F. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. Carcinogenesis 12: 1839–1845 (1991).
10. Alexander, J. A. and Wallin, H. Metabolic fate of heterocyclic amines. In Mutagens in Food: Detection and Prevention (H. Hayatsu, Ed.), CRC Press, Boca Raton, FL, 1991, pp. 143–156.
11. Turesky, R. J., Skipper, P. L., Tannenbaum, S. R., Coles, B., and Ketterer, B. Sulfamate formation is a major route of detoxification of 2-amino-3-methylimidazo[4,5-f]quinoxaline in the rat. Carcinogenesis 7: 1483–1485.
12. Luk, H. J., Spratt, T. E., Vanrek, M. T., Roland, S. F., and Weisburger, J. H. Identification of sulfate and glucuronic acid conjugates of the 5-hydroxy derivative as major metabolites of 2-amino-3-methylimidazo[4,5-f]quinoxaline in rats. Cancer Res. 49: 4407–4411 (1989).
13. Skipper, P. L., and Tannenbaum, S. R. Protein adducts in the molecular dosimetry of chemical carcinogens. Carcinogenesis 11: 507–518 (1990).
14. Flammang, T. J., Talaska, G., Chu, D. J. Z., Lang, N. P., and Kadlubar, F. F. \(N\)-acetyltransferase polymorphism and arylamine toxicity: relationship to \(O\)-acylation reactions. In: Intermediary Xenobiotic Metabolism in Animals. Methodology, Mechanisms, and Significance (D. H. Hutson, J. Caldwell, and G. D. Paulson, Eds.), Taylor and Francis, London, 1988, pp. 155–177.
15. Felton, J. S., Knize, M. G., Shen, N. H., Wu, R., and Becher, G. Mutagenic heterocyclic imidazourines in cooked foods. In: Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarones (C. M. King, L. J. Romano, and D. Schuetzle, Eds.), Elsevier, New York, 1988, pp. 73–83.
16. Felton, J. S., Knize, M. G., Shen, N. H., Andresen, B. D., Bjedenas, L. F., and Hatch, F. T. Identification of the mutagens in cooked beef. Environ. Health Perspect. 67: 17–14 (1986).
17. Day, B. W., Naylor, S. N., Gan, L. S., Sahali, Y., Nguyen, T. T., Skipper, P. L., Wishonk, J. W., and Tannenbaum, S. R. Molecular dosimetry of polycyclic aromatic hydrocarbon epoxides and dipol epoxides via hemoglobin adducts. Cancer Res. 50: 4601–4608 (1990).
18. Lynch, A. M., Murray, S., Boobis, A. R., Davies, D. S., and Goodnerham, N. J. The measurement of MelQx adducts with mouse haemoglobin in vivo and in vivo: implications for human dosimetry. Carcinogenesis 12: 1067–1072 (1991).
19. Butler, M. A., Iwasaki, M., Guengerich, F. P., and Kadlubar, F. F. Human cytochrome P-450sa (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and \(N\)-oxidation of carcinogenic arylamines. Proc. Natl. Acad. Sci. U.S.A. 86: 769–7700 (1989).
20. Shimada, T., Iwasaki, M., Martin, M., and Guengerich, F. P. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by unmu gene response in Salmonella typhimurium TA 1535/pSK1002. Cancer Res. 49: 3218–3228 (1989).
21. Kato, R. Metabolic activation of mutagenic heterocyclic aromatic amines from protein pyrolysates. CRC Crit. Rev. Toxicol. 16: 307–348 (1986).
22. Kaderlik, K., Turesky, R. J., Teitel, C. H., Lang, N. P., and Kadlubar, F. F. Metabolic pathways for heterocyclic amines in humans in relation to colon carcinogenesis. Proc. Am. Assoc. Cancer Res. 32: 119 (1991).
23. Snyderwine, E. G., Adamson, R. H., Welti, D. H., Richli, U., Thorgeirsson, S. S., Würzner, H. P., and Turesky, R. J. Metabolism of 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQ) in the monkey. Proc. Am. Assoc. Cancer Res. 32: 121 (1991).
24. Gross, G. A., and Günter, A. Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. J. Chromatogr. 592: 271–278 (1992).