Macromolecular adduct formation and metabolism of heterocyclic amines in humans and rodents at low doses

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Abstract

2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) are heterocyclic amines formed during the cooking of meat and fish. Both are genotoxic in a number of test systems and are carcinogenic in rats and mice. Human exposure to these compounds via dietary sources has been estimated to be under 1 \(\mu\)g/kg body wt. per day, although most laboratory animal studies have been conducted at doses in excess of 10 mg/kg body wt. per day. We are using accelerator mass spectrometry (AMS), a tool for measuring isotopes with attomole sensitivity, to study the dosimetry of protein and DNA adduct formation by low doses of MeIQx and PhIP in rodents and comparing the adduct levels to those formed in humans. The results of these studies show: 1, protein and DNA adduct levels in rodents are dose-dependent; 2, adduct levels in human tissues and blood are generally greater than in rodents administered equivalent doses; and 3, metabolite profiles differ substantially between humans and rodents for both MeIQx and PhIP, with more \(N\)-hydroxylation (bioactivation) and less ring oxidation (detoxification) in humans. These data suggest that rodent models do not accurately represent the human response to heterocyclic amine exposure. © 1999 Published by Elsevier Science Ireland Ltd.

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1. Introduction

Since the discovery of mutagenic compounds in cooked and charred meats by Sugimura et al. [1] over 20 years ago, a number of laboratories around the world have devoted significant effort to determine if these agents are human cancer risk factors. To date, approximately 19 heterocyclic amines (HCAs) have been shown to form in meat and other products upon heating [2] and all HCAs tested for carcinogenicity in either rodents or non-human primates have been found to be carcinogenic [3]. Concern over the health significance of these compounds is supported by evidence that human exposure does occur [4,5]. Furthermore, several studies have shown an association between consumption of fried or grilled meats and meat-based gravies with colorectal cancer [6–9] and breast cancer [10,11], although others have not [12].
While a number of estimates have been made on the risk these agents may pose to humans [13] very little data are actually available on the toxicokinetics, metabolism and DNA binding of these compounds in humans to support these estimates. Human liver microsomes have been shown to $N$-oxidize the HCAs at similar, if not greater rates than found in laboratory animals [14,15]. Hepatic and non-hepatic tissues are capable of conjugating some of the $N$-oxidized HCAs to sulfate and acetate esters; metabolites capable of covalently modifying DNA [16,17]. Urine analysis of humans eating fried meats has demonstrated that the HCAs are extensively metabolized, and several of the metabolites have been identified [18]. Some evidence has shown that DNA adducts are present in people eating cooked meat [14,19]. However, the major pathways of metabolism, the dosimetry and quantitative relationships between humans and the laboratory animals on which HCA tumorigenic risk is based, remain unknown. Hence there are many remaining issues to be addressed. These include determining the ability of humans to bioactivate HCAs at the low doses encountered in the environment and investigating if DNA or protein adducts can be consistently detected in target and non-target tissues at these levels. Furthermore, it is important to establish how metabolism and the levels of macromolecular adducts compare among species, how the various metabolites and adducts relate to each other and whether adducts or metabolites will be useful indicators of risk or are related to phenotype or genotype (see Lang et al., these proceedings).

We have begun pilot studies to explore whether metabolites and macromolecular adducts of HCAs can be measured in humans and how they compare to rodents at well-defined dietary-relevant doses. Our approach is to utilize $^{14}$C-labeled HCAs and the technique of accelerator mass spectrometry (AMS) to quantify adducts, and liquid scintillation counting in conjunction with LC-MS to quantify and characterize the metabolites. AMS is a technique that measures attomole levels of $^{14}$C-labelled agents with high precision, and which we originally applied to the study of xenobiotic metabolism and DNA binding [20–22]. Using this technology, we have been able to administer ng–μg HCA doses having activities in the nCi–μCi range to animals and humans. The amounts of HCAs in these doses are equivalent to the consumption of a few servings of cooked meat and the radioisotope exposures are equivalent to less than 1% of a person’s average annual exposure to all sources of natural ionizing radiation. Thus this technology allows us to study the processing and macromolecular damage caused by the HCAs at relevant doses in animals and safely in humans.

2. Methods

2.1. Animal studies

We have focused on 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) since these are the most mass abundant HCAs in Western-type diets. Previous studies in rodents have shown that MeIQx and PhIP are absorbed and bioavailable to tissues over a large dose range. These doses span the human exposure level and the much higher tumor bioassay doses. Over this dose range the amount of HCA available to the tissues is generally a linear function of administered dose [21,23–26]. Similarly, dose-dependent levels of DNA adducts are found in liver, colon, breast and prostate [23,27] and protein adducts in peripheral blood [28] are present in rodents administered these compounds at doses ranging from 10 ng–100 mg/kg body weight. These data show that HCAs are bioactivated at low doses in these animals and that some bioactive metabolites enter the circulation to reach the tissues. Thus the HCAs are metabolized to toxic metabolites and are available to the tissues at both the doses which induce tumors in laboratory animals and at doses which humans are exposed to in the diet. The internal dose and target cell bioactive HCA dose is generally a linear function of administered dose in these animals.

2.2. Human studies

We have now conducted pilot studies to determine if macromolecular adducts can be quantitatively measured at dietary-relevant doses in humans. These studies should show how adduct levels relate to metabolite profiles, metabolic polymorphisms and the animal models discussed above. The studies have been approved by the human subjects review boards.
at the institutions involved in this work. Human volunteers diagnosed with colon cancer were enrolled into the studies after being informed of the risks and providing written consent. For the MeIQx study, subjects were administered a gelatin capsule containing either 21 mg (five subjects, approx. 0.3 mg/kg body wt.) or 228 mg (two subjects, approx. 3 mg/kg body wt.) [14C]MeIQx 3.5±6 h prior to surgical resection of their tumors at York District Hospital, UK. Urine, blood and tissue samples were collected and stored frozen until processing. DNA was extracted from colon tissue and albumin and hemoglobin were isolated from blood and analyzed by AMS using previously reported methods [28]. Liquid scintillation counting (LSC) and LC-MS was used to quantify and characterize urinary metabolites in samples from the 21 mg dose. A similar study design is underway with PhIP, except that each subject has been administered a gelatin capsule containing 70 mg (n = 2) or 84 mg (n = 3) [14C]PhIP (approximately 1 mg/kg body wt.) 48–72 h prior to surgery at the University of Arkansas Cancer Center, Little Rock, AR. In addition to adduct analysis, plasma clearance kinetics and metabolite profiles were determined.

3. Results

3.1. Clearance kinetics

Both MeIQx and PhIP were rapidly detected in the urine of the human subjects after oral administration. Between 25 and 90% of the dose was eliminated in the urine within 24 h [29,30]. Peak PhIP levels in the plasma (approx. 1 ng/ml plasma) were attained within 2 hours of exposure. It was not possible to calculate clearance kinetics for MeIQx, although based upon urine and plasma clearance, the half-life of PhIP was determined to be between 2 and 4 h in these subjects.

3.2. Plasma protein adducts

MeIQx and PhIP formed albumin and hemoglobin adducts in the peripheral blood of the human subjects, with albumin adduct levels consistently greater than hemoglobin adduct levels. For MeIQx, the mean adduct level per unit dose (fmol MeIQx/g protein per pmol MeIQx per kg body wt.) was 17.37 and 0.85 for albumin and hemoglobin, respectively [28].

### Table 1

HCA-DNA and -protein adduct levels per unit dose in the colon and blood of humans and rodents

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample type</th>
<th>Compound</th>
<th>Mean adduct level per unit dose</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Albumin</td>
<td>MeIQx</td>
<td>17.37</td>
<td>9.15, 32.91c</td>
<td>7</td>
</tr>
<tr>
<td>Human</td>
<td>Albumin</td>
<td>PhIP</td>
<td>11.42</td>
<td>5.04</td>
<td>5</td>
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<tr>
<td>Human</td>
<td>Colon DNA</td>
<td>MeIQx</td>
<td>2.24</td>
<td>1.84</td>
<td>7</td>
</tr>
<tr>
<td>Human</td>
<td>Colon DNA</td>
<td>PhIP</td>
<td>1.13</td>
<td>0.67</td>
<td>5</td>
</tr>
<tr>
<td>Human</td>
<td>Hemoglobin</td>
<td>MeIQx</td>
<td>0.85</td>
<td>0.19, 3.62c</td>
<td>7</td>
</tr>
<tr>
<td>Human</td>
<td>Hemoglobin</td>
<td>PhIP</td>
<td>0.20</td>
<td>0.09</td>
<td>5</td>
</tr>
<tr>
<td>Rat</td>
<td>Albumin</td>
<td>MeIQx</td>
<td>2.16</td>
<td>1.13, 4.32c</td>
<td>9</td>
</tr>
<tr>
<td>Rat</td>
<td>Albumin</td>
<td>PhIP</td>
<td>3.26</td>
<td>0.86</td>
<td>3</td>
</tr>
<tr>
<td>Rat</td>
<td>Colon DNA</td>
<td>MeIQx</td>
<td>0.94</td>
<td>0.19</td>
<td>6</td>
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<tr>
<td>Rat</td>
<td>Colon DNA</td>
<td>PhIP</td>
<td>0.11</td>
<td>0.09</td>
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</tr>
<tr>
<td>Rat</td>
<td>Hemoglobin</td>
<td>MeIQx</td>
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<td>0.05, 1.36c</td>
<td>6</td>
</tr>
<tr>
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<td>PhIP</td>
<td>0.51</td>
<td>0.07</td>
<td>3</td>
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<td>Mouse</td>
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<td>MeIQx</td>
<td>0.79</td>
<td>0.26</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1 is captioned: Units are fmol HCA/g sample per pmol dose/kg body weight

* Mean levels (±SD) of blood protein adducts and colon DNA adducts in human subjects and rodents administered [14C]MeIQx or [14C]PhIP. The [14C]HCAs were administered to the human subjects in a gelatin capsule. Colon tissue and blood was collected at 3.5–6 h post dose administration for MeIQx. Colon tissue was collected 48–72 h post dose for PhIP, and albumin and hemoglobin adduct levels calculated using the 4 h blood samples. Rodents were administered the [14C]HCAs by gavage and were sampled at 4.5 h for MeIQx and 6 h for PhIP. Radiocarbon levels in the samples were determined by AMS. MeIQx data from [26] and [28].
forms that will bind to DNA in the colon, a site where tumors have been seen in rodent bioassays and a site associated with cancers in frequent meat eaters or those preferring well-cooked meats [7,9]. Human colon DNA adduct levels in the normal mucosa of the subjects 3.5–6 h after administration of [14C]MeIQx were dose-dependent, with adduct levels in the range 0.9–1.2 DNA adducts/10^9 nucleotides at 3 µg/kg body wt. dose (n = 2) and 0.02–0.04 DNA adducts/10^9 nucleotides at 0.3 µg/kg body wt. dose (n = 5) [26,31]. PhIP–DNA adduct levels in the normal colon mucosa were in the range 0.03–0.13 adducts/10^9 nucleotides (n = 5) 48–72 h after administration of 1 µg/kg body wt. [14C]PhIP. In comparison, data obtained in rodents was significantly lower than detected in the human colon samples (Table 1).

3.5. Comparative metabolism

Both PhIP and MeIQx are extensively metabolized in humans and rodents. Analysis of urine from the subjects administered [14C]MeIQx shows the presence of several metabolites: N^2-(3,8-dimethylimidazo[4,5-f]quinoxalin-2-yl)sulfamic acid (MeIQx-N^2-SO3), N^2-[(β-1-glucosiduronyl)-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx-N^2-Gl), 2-amino-8-hydroxymethyl-3-methylimidazo[4,5-f]quinoxaline (8-CH_2OH-MeIQx), N^2-(β-1-glucosiduronyl)-N-hydroxy-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (NH OH-MeIQx-N^2-Gl), MeIQx and an unidentified metabolite [29]. The primary metabolites were the unidentified metabolite and the NHOH-MeIQx-N^2-Gl. The PhIP metabolites identified in human urine are shown in Fig. 1. The major metabolite is the NHOH-PhIP-N^2-Gl. In contrast to the humans, the major pathways for metabolism of MeIQx and PhIP in rodents involves ring hydroxylation at the C-5 carbon for MeIQx and at the 4′-carbon of the phenyl ring of PhIP [32–36].

4. Discussion

This is a summary of the initial findings from two pilot studies to determine the metabolism and macro-molecular adduct formation of HCAs in humans at well-defined dietary-relevant doses. Where possible, results from these studies have been compared to data obtained in rodents. These studies show that in
humans the HCAs PhIP and MeIQx are rapidly absorbed and eliminated when administered orally at low dose and are bioavailable to colon tissue. Similarly, these compounds are absorbed and bioavailable to the tissues of rodents when administered at doses approximating human dietary exposure. In both rodents and humans, these HCAs are bioactivated at low dose and form both blood protein and colon DNA adducts, suggesting that bioactivation occurs and that some proportion of these bioactive metabolites circulate. Future work needs to be undertaken to identify the proximate genotoxins of these compounds which result in target tissue damage. Importantly, PhIP and MeIQx generally form greater levels of both blood protein and colon DNA adducts in humans relative to rodents, which may be the result of a greater capacity of humans to bioactivate these compounds. Furthermore, a larger proportion of N-hydroxylated metabolites (MeIQx-NHOH-N2-Gl, PhIP-NHOH-N2-Gl and PhIP-NHOH-N3-Gl) were seen in human urine and plasma relative to rodents and humans also appeared to have a lower capacity to ring hydroxylate
(a detoxification pathway) these compounds relative to rodents. Our findings are supported by in vitro studies showing human tissue cytosols and microsomal extracts have a greater capacity to N-hydroxylate HCAs than do rodents [14,15]. However, bile analysis in humans has not been carried out and this may affect our conclusions regarding HCAs and colon cancer. Further work is currently underway to expand the number of human subjects in these studies, to relate metabolite profiles to adduct levels and to metabolic polymorphisms (see Lang et al., these proceedings). These studies will establish if HCA metabolites or adducts may be useful markers of the bioactive HCA dose to the tissues, and may also show if individual metabolic profiles relate to genetic capacity to bioactivate these compounds.

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References


