Urinary Excretion of 2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline in White, Black, and Asian Men in Los Angeles County

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Abstract

The heterocyclic aromatic amines produced by high temperature cooking of foods containing creatin(in)e and amino acids (such as beef, pork, poultry and fish) are a class of potent animal carcinogens and have been implicated indirectly in human colon and pancreas carcinogenesis. We studied the urinary excretion of a mutagenic heterocyclic aromatic amine compound, 2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx), among 47 black, 41 Asian (Chinese or Japanese), and 43 non-Hispanic white (white) male residents of Los Angeles County by quantitative analysis of total free and conjugated MeIQx in pooled overnight urine collections. Significant interracial differences were observed. Geometric mean level in blacks was 1.3- and 3.0-fold higher than that in Asians and whites, respectively. Urinary level of MeIQx was positively associated with intake frequencies of bacon, pork/ham and sausage/luncheon meats among study subjects, consistent with the observation that in Los Angeles, blacks had the highest consumption of these three food groups among the three races. Among men in Los Angeles County, the incidence rates of pancreas and colon cancers, which have been shown to be positively related to intake of fried meats, are 50% and 20% higher in blacks relative to Asians and whites, respectively. Our results are, therefore, consistent with the hypothesis that exposure to heterocyclic aromatic amines is related to risk of pancreas and colon cancers, and may in part explain the higher incidence among blacks relative to Asians and whites in Los Angeles.

Introduction

The HAAs produced by high temperature cooking of foods containing creatin(in)e and amino acids (such as beef, pork, poultry, and fish) are a class of potent animal carcinogens to which people are exposed frequently and in significant concentrations (1). As such, they are potential etiological agents for one or more human cancers. A number of epidemiological studies have implicated foods containing HAAs as playing a role in human colon and pancreas cancers (2–7). No studies have yet been conducted between the incidence of any specific cancer and directly determined exposure to HAAs.

The decline (or increase) of specific cancer incidence rates in ethnically homogeneous populations as a result of migration of the population from one cultural/geographical region to another has been useful in identifying etiological factors. Such studies, for instance, have been instrumental in establishing exposure to environmental agents as an important determinant of risk for cancers of the stomach, liver, breast, and colon (8). Although less powerful, the study of heterogeneous populations with cancer incidence rates that covary with ethnic or racial identity can also be fruitful in identifying carcinogenic agents the exposure levels of which differ among the studied populations.

The Los Angeles County population is ethnically diverse, including large subpopulations of Chinese, Japanese, blacks, and Hispanic and non-Hispanic whites. There also is a population-based cancer surveillance program in place in Los Angeles County, which has characterized the incidence and mortality rates for cancers of different types among the different ethnic groups (9). The combination of these factors makes the Los Angeles County population a suitable one in which to test the effect of ethnically/culturally mediated differences in exposure to or metabolism of suspected carcinogenic agents on cancer outcome. In this paper we compare urinary levels of a mutagenic HAA compound, MeIQx, among black, Asian (Chinese or Japanese), and white men of Los Angeles County.

Materials and Methods

Subjects. Detailed characteristics of our study subjects have been described elsewhere (10). Briefly, they were male residents of Los Angeles County who were over the age of 35 years and were either non-Hispanic white (white; n = 43), black (n = 47) or Asian (n = 41; 17 Chinese, 24 Japanese). By design, 69 (53%) subjects were lifelong non-

1 The abbreviations used are: HAA, heterocyclic aromatic amine; MelQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; PBS, phosphate-buffered saline.
smokers; the remaining 62 subjects were current cigarette smokers of varying intensity. Seventy-six of the 131 study subjects were participants of a cross-sectional survey among white, black, and Asian male residents of Los Angeles County, in which current dietary information was collected through a self-administered questionnaire (10). Each subject was asked to collect an overnight urine sample (ending with the first morning void) after drinking coffee prepared from 2 packets of Nescafe Instant Coffee (about 70 mg of caffeine) between 3 and 6 p.m. the previous day. All urine specimens were acidified (400 mg of ascorbic acid/20 ml of urine) within 24 h of collection and subsequently stored at −20°C until analysis.

**Determination of MeIQx and Creatinine in Urine.** Quantitative analysis of free plus conjugated MeIQx was performed as described in detail elsewhere (11). Briefly, urine specimens (15 ml) were acidified by the addition of 6 N HCl (3 ml) and heated at 70°C for 6 h. This procedure liberates MeIQx from its conjugates with sulfuric acid (12) and β-glucuronid acid (data not shown). After addition of Me-2H3I, IQx (1 ng), the samples were neutralized with 6 N NaOH (3 ml) and Na2CO3 (1 g) and extracted twice with 2 volumes of CH3CO2C2H5. The CH3CO2C2H5 phase was chilled to freeze out residual aqueous phase and then extracted with 0.1 N HCl. The acid extracts were dried with a Speed-Vac concentrator and reconstituted in PBS. MeIQx was isolated from the PBS solutions by immunoadsorption chromatography (13) preceded by passage of the PBS solution through a column of Tri-blocked CNBr-activated Sepharose 4B. MeIQx was eluted from the columns with 1 N CH3CO2H, which was subsequently removed by evaporation. The residue was partitioned between CH3CO2C2H5 and 1 N Na2CO3. The CH3CO2C2H5 phase was retained and the solvent was removed by evaporation under a stream of N2. The di-di-(3,5-trifluoromethyl)benzyl derivative of MeIQx was prepared using the method of Murray et al. (14).

The derivatives were analyzed using a Hewlett-Packard 5987 gas chromatography-mass spectrometry instrument equipped with a chemical ionization source and negative ion detection. Gas chromatography was performed on a Hewlett-Packard HP-1 column (12.5 m x 0.2 mm) with Helium as carrier gas. Negative ions at m/z 438 and m/z 441 were monitored and quantitation was accomplished by comparison of the integrated peak areas, assuming equal detector response for each ion. Blank urines were analyzed under the same conditions to determine the amount of signal at m/z 438 contributed by the internal standard.

Creatinine levels in the 131 urine specimens were determined using standard methods at the Clinical Laboratory of the Kenneth Norris, Jr. Cancer Hospital. As a means of adjusting for the varying concentration of urine among study subjects, we used the MeIQx:creatinine ratio as the biomarker for MeIQx exposure *in vivo*.

**Statistical Analysis.** The distribution of the MeIQx:creatinine ratio was markedly skewed in our study population; therefore, formal statistical testing was performed on logarithmically transformed values of the ratio and geometric (as opposed to arithmetic) mean values are presented. We used the analysis of variance method to compare log values of the MeIQx:creatinine ratio across the three ethnic groups. We used the $\chi^2$ test of association to examine the relationship between the MeIQx:creatinine ratio and level of education, alcohol consumption, and current frequencies of intake of 11 selected foods among the 76 subjects in whom both sets of information were available. The $\chi^2$ test of association was also used to examine possible differences in frequencies of intake of the above foods among the 519 white, black, and Asian men who were the primary source of our study participants (see "Subjects" for details). All $P$ values quoted are two-sided.

**Results**

Since MeIQx was determined after acid treatment of urine specimens, the values of our measurement represent the sum of free and conjugated MeIQx. These values range from undetectable (<1 pg/ml of urine) to 312 pg/ml of urine.

Fig. 1 presents the individual values of urinary MeIQx expressed in nanograms of MeIQx/gram of creatinine for the 131 study participants by race. Table 1 presents the geometric mean levels, which were highest in blacks (3.78), intermediate in Asians (2.81), and lowest in whites (1.24). The difference in mean levels across the three ethnic groups was statistically significant ($P = 0.0009$). Table 1 also lists the distribution of MeIQx levels across the three races. The two cutpoints, 2.5 and 5.0 ng/g creatinine, were chosen to separate the positive subjects into approximate tertiles. Only 19% of black subjects had undetectable MeIQx in urine versus 63% in whites. On the other hand, 36% of black subjects but only 7% of white subjects had levels of MeIQx exceeding 5.0 ng/g of creatinine.
petitioned questionnaire; comparable information on the remaining 55 subjects.

For bacon, frequency between the three races was most pronounced. Of these three foods, the difference in intake

groups that exhibited positive association with urinary level

tently showed the highest intake frequencies of bacon, consis-
ticantly significant (P = 0.01).

Table 3 compares, between subjects exhibiting low versus high levels of urinary MelQx, the intake frequencies of six foods which have the potential of generating appreciable amounts of HAA during cooking. Intakes of bacon, pork/ham, and sausage/luncheon meat were positively associated with the level of urinary MelQx. The association was especially pronounced for bacon. Consistent with the above observations, Los Angeles blacks showed the highest consumption of bacon, for bacon. Consistent with the above observations, Los Angeles blacks showed the highest consumption of bacon, pork/ham, and sausage/luncheon meat.

Perspective and commentary

Results and discussion

Table 2 compares, between subjects exhibiting low versus high levels of urinary MelQx, the intake frequencies of six foods which have the potential of generating appreciable amounts of HAA during cooking. Intakes of bacon, pork/ham, and sausage/luncheon meat were positively associated with the level of urinary MelQx. The association was especially pronounced for bacon. Consistent with the above observations, Los Angeles blacks showed the highest consumption of bacon, pork/ham, and sausage/luncheon meat.

A second comparison is with the data of Lynch et al. (16), who found that their subjects excreted about 2% of the MelQx present in cooked meat, leading to levels of about 10 ng excreted following a meal containing relatively high amounts of MelQx (i.e., 530 ng MelQx in 240 g fried beef). The average of positive values within our three races (whites 3.3, Asians 8.3, and blacks 12.7 ng/g creatinine) would extrapolate to a total of 4–12 ng in the 8-h postprandial period used by Lynch et al. (16). However, these values must be adjusted downward by a factor of 5–10 to estimate the amount of free MelQx present, and they then become considerably smaller than the values of Lynch et al. (16). Few if any of our subjects would be expected to have had a recent meal with a MelQx content of 530 ng or greater.

Our study indicates that blacks have considerably higher levels of urinary MelQx compared to Asians and whites in Los Angeles County. We further noted that MelQx levels in these subjects were positively associated with their intake frequencies of bacon, pork/ham, and sausage/luncheon meats. The association was especially pronounced for bacon. Consistent with the above observations, Los Angeles blacks showed the highest consumption of bacon, pork/ham, and sausage/luncheon meats relative to their white and Asian counterparts. Again, bacon had the greatest difference in intake frequency among the three races. Our findings are not surprising; fried bacon has been shown

Table 1 Levels of urinary MelQx* among study subjects

<table>
<thead>
<tr>
<th>Foods</th>
<th>Black</th>
<th>Asian</th>
<th>White</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.D.</td>
<td>9 (19)</td>
<td>15 (37)</td>
<td>27 (63)</td>
<td>51 (39)</td>
</tr>
<tr>
<td>N.D. &lt; R = 2.5</td>
<td>14 (30)</td>
<td>8 (19)</td>
<td>7 (16)</td>
<td>29 (22)</td>
</tr>
<tr>
<td>2.5 &lt; R ≤ 5.0</td>
<td>7 (15)</td>
<td>9 (22)</td>
<td>6 (14)</td>
<td>22 (17)</td>
</tr>
<tr>
<td>5.0 &lt; R</td>
<td>17 (36)</td>
<td>9 (22)</td>
<td>3 (7)</td>
<td>29 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (100)</td>
<td>41 (100)</td>
<td>43 (100)</td>
<td>131 (100)</td>
</tr>
</tbody>
</table>

Geometric mean level 3.78 2.81 1.24 2.48

*Expressed in ng/g of creatinine.

Table 2 Frequencies of intake of selected foods according to levels of MelQx in urine among study subjects

<table>
<thead>
<tr>
<th>Foods</th>
<th>Level of MelQx*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n = 45)</td>
</tr>
<tr>
<td>Bacon (1)</td>
<td>16</td>
</tr>
<tr>
<td>Beef</td>
<td>60</td>
</tr>
<tr>
<td>Fish</td>
<td>23</td>
</tr>
<tr>
<td>Pork/ham (2)</td>
<td>27</td>
</tr>
<tr>
<td>Poultry</td>
<td>58</td>
</tr>
<tr>
<td>Sausage/luncheon meat (3)</td>
<td>29</td>
</tr>
</tbody>
</table>

*Seventy-six of our 131 study subjects were participants in an earlier survey in which current dietary information was collected through a self-administered questionnaire; comparable information on the remaining 55 subjects was unavailable.

Table 3 Frequencies of intake of selected foods among white, black and Asian men of Los Angeles County

<table>
<thead>
<tr>
<th>Foods</th>
<th>Blacks (n = 83)</th>
<th>Asians (n = 267)</th>
<th>Whites (n = 169)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacon (1)</td>
<td>33</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Beef</td>
<td>45</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>Fish</td>
<td>10</td>
<td>33</td>
<td>17</td>
</tr>
<tr>
<td>Pork/ham (2)</td>
<td>28</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Poultry</td>
<td>55</td>
<td>43</td>
<td>54</td>
</tr>
<tr>
<td>Sausage/luncheon meat (3)</td>
<td>30</td>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>1, 2, or 3 b</td>
<td>73</td>
<td>57</td>
<td>62</td>
</tr>
</tbody>
</table>

*Numbers represent the percentage of subjects in each racial/ethnic group whose intake frequencies of the three food groups were 52 or more times/year.

b We add up the intake frequencies of bacon, pork/ham, and sausage/luncheon meat.

Discussion

Our MelQx readings may be compared to data obtained in two previous studies conducted by others, although not directly, since we measured the sum of MelQx and its conjugates rather than just free MelQx and the urine collection procedures were not the same. Ushiyama et al. (15) determined 24-h excretion of free MelQx by 10 Japanese subjects consuming their normal, unrestricted diet and found values ranging from 11 to 47 ng. Our values for Japanese subjects range from nondetectable to 63 ng/g creatinine with an arithmetic mean of 6.6. On a 24-h rather than per gram creatinine basis, the numbers would be 50–100% higher, making them comparable to the data of Ushiyama et al. (15), except that they are uncorrected for the free MelQx conjugates ratio.

modifying HAA metabolism (salad greens, tomatoes, green vegetables, yellow or orange vegetables, and white or other vegetables). All results were negative.
to possess one of the highest levels of HAA among all cooked foods examined to date (17, 18).

Although frequency of bacon consumption was strongly associated with urinary MelQx, it does not explain all the differences observed among the three groups since Asians had intermediate mean MelQx but consumed bacon least frequently. Frequent consumption of bacon may be more a marker of a general preference for foods cooked in such a way as to be high in HAA content. Recent studies have clarified the factors that increase the formation of HAA during cooking (19), which include cooking on a heated surface (sometimes referred to as grilling), high cooking temperature, and extensive charring or crustling of the meat surface. All of these apply to bacon. Exceeding the time and temperature parameters that yield maximal HAA content in the meat serves principally to transfer the HAA to the grill residues, which are often used further to make sauces and gravies, or for flavoring. MelQx levels in some foods can thus exceed those in bacon if they are cooked in the appropriate manner.

Several cancers occur more frequently among black males living in Los Angeles County than among white or Asian males. These include multiple myeloma, cancer of the oral cavity, esophagus, larynx, lung, prostate, colon, and pancreas (9). Multiple myeloma has a poorly understood etiology, but there is little evidence to associate it with diet. Cancers of the upper alimentary canal and respiratory system are linked strongly to alcohol and tobacco consumption. For the last three cancers, of the prostate, colon, and pancreas, it has long been suggested that diet plays a role (20), with dietary fat most frequently implicated. Fat consumption, however, is not independent of meat consumption, and it could be that both fat and meat intake serve principally as surrogate measures for exposure to HAA. Animal experiments also support the possibility of a role for HAAs in these cancers (1, 21).

Some aspects of the comparative epidemiology of cancer in the United States and Japan support a role for HAAs in the etiology of colon and pancreas cancers but not prostate cancer (22, 23). From the early 1950s until 1985, the per capita consumption of red meat and fats/oils in Japan increased from 8 to 105 g (1200%) and from 5 to 40 g (700%), respectively. In the United States the comparable increases were 39% and 75%, respectively. In the same period, pancreas cancer mortality rates for Japanese males rose from 2/100,000 to match the United States white rate of 8/100,000, which had remained nearly constant. Similarly, colon cancer mortality rate rose from 2/100,000 to 8/100,000 among Japanese males for the same time period, compared to 15/100,000 among United States white males. Mortality from cancer of the prostate, in contrast, has remained very low in Japan compared to the United States white rate (about 2/100,000 versus 14/100,000).

In summary, we have observed significant differences in the excretion of free plus conjugated MelQx between different racial/ethnic groups living in Los Angeles County. Our data suggest that the greater excretion of MelQx among blacks relative to Asians and whites is the result of greater dietary exposure to HAA through ingestion of fried meats such as bacon. Among men in Los Angeles County, the incidence rates of pancreas and colon cancers, which have been shown to be positively related to the intake of fried meats (2–7), are 50% and 20% higher in blacks relative to Asians and whites, respectively. Our results are, therefore, consistent with the hypothesis that exposure to HAA is related to the risk of pancreas and colon cancers, and may in part explain the high rates in blacks relative to Asians and whites in Los Angeles.

Acknowledgments
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