Influence of Coffee Intake on Urinary Hippuric Acid Concentration

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Abstract: Intake of foods and drinks containing benzoic acid influences the urinary hippuric acid (HA) concentration, which is used to monitor toluene exposure in Japan. Therefore, it is necessary to control the intake of benzoic acid before urine collection. Recently, some reports have suggested that components of coffee, such as chlorogenic, caffeic, and quinic acids are metabolized to HA. In this study, we evaluated the influence of coffee intake on the urinary HA concentration in toluene-nonexposed workers who had controlled their benzoic acid intake, and investigated which components of coffee influenced the urinary HA concentration. We collected urine from 15 healthy men who did not handle toluene during working hours, after they had consumed coffee, and we measured their urinary HA concentrations; the benzoic acid intake was controlled in these participants during the study period. The levels of chlorogenic, caffeic, and quinic acids in coffee were analyzed by LC-MS/MS. Urinary HA concentration increased significantly with increasing coffee consumption. Spectrophotometric LC-MS/MS analysis of coffee indicated that it contained chlorogenic and quinic acids at relatively high concentrations but did not contain benzoic acid. Our findings suggest that toluene exposure in coffee-consuming workers may be overestimated.

Key words: Biological monitoring, Hippuric acid, Toluene, Coffee intake, Chlorogenic acid

Introduction

In Japan, health control of workers is accomplished by controlling work environment conditions and improving work practice management and health care. Urinary hippuric acid (HA) levels are measured through periodic monitoring of workers exposed to toluene during medical examination, as mandated by the Ministry of Health, Labour and Welfare1). However, toluene is metabolized to HA via benzoic acid in the human body. Therefore, the dietary intake of benzoic acid, which is present in certain acidic foods (e.g., berries, plums, cranberries, and prunes) and used in food preservatives, also influences urinary HA concentration. Hence, it is necessary to develop methods that eliminate the effect of dietary HA and other environmental influences that contribute to the background concentration of HA2).

The Ministry of Health, Labour and Welfare of Japan has established a 3-tier classification system (distributions 1, 2, and 3) based on urinary HA level in workers (Table 1). “Distribution 3” consists of workers exposed to concentrations that are more than the 1988–1989
biological exposure indices released at the American Conference of Industrial Hygienists (ACGIH)\textsuperscript{3}). The HA concentration in most workers is usually within “Distribution 1” under appropriate control of toluene exposure, especially when the intake of benzoic acid is controlled for at least 1 day before urine collection. Nevertheless, elevated urinary HA concentrations are sometimes observed even when the intake of benzoic acid is strictly controlled\textsuperscript{4}).

It was recently reported that the consumption of coffee results in the elevation of urinary hippuric acid concentration\textsuperscript{5–7)}, and it was indicated that the components of coffee (in particular, chlorogenic, caffeic, and quinic acids) are metabolized to HA\textsuperscript{6}). Munaka \textit{et al.}\textsuperscript{8)} reported that the consumption of green tea and/or coffee can result in an overestimation of urinary HA concentrations and cause false-positive results during the biological monitoring of workers exposed to low doses of toluene.

In this study, we evaluated the influence of coffee intake on urinary HA concentration when benzoic acid intake was controlled in toluene-nonexposed workers, and analyzed the components in coffee that influenced urinary HA concentration.

\section*{Materials and Methods}

\textit{Participants and urine collection}

The participants in this study were 15 healthy male medical staff (age range, 29–56 yr) who had not handled toluene. This study was approved by the Ethics Committee of Tokyo Rosai Hospital (approval number 20-6). All subjects gave informed consent.

Urine was collected on days when the participants did not drink coffee or had 1, 2, or 3 cups of coffee (per day). Samples were collected every 3–4 d, and in some cases, every other day. The participants consumed the drip coffee that is readily available in Japan. They were not permitted to consume alcohol or foods/drinks containing benzoic acid for at least 1 d before urine collection. Moreover, they were instructed to drink only coffee or water during working hours.

On the urine collection day, they could drink coffee at any time during working hours. Similar to the procedure followed in the biological monitoring of toluene, we collected urine at the end of the working day. The urine samples were preserved at 4°C in a refrigerator before further analysis.

\textit{Measurement of urinary HA and creatinine concentrations}

Urinary HA was measured by high-performance liquid chromatography (HPLC) within 1 wk after collection in the laboratory of Mitsubishi Chemical Medience Corporation (Tokyo, Japan) following the procedure reported by Ogata \textit{et al.}\textsuperscript{9}). The analytical quality of HA analysis in this laboratory was certificated by National Federation of Industrial Health Organization in Japan\textsuperscript{10}).

We preserved urine samples at 4°C until HA analysis. Although we did not examine the stability of HA in these samples, the National Institute for Occupational Safety and Health (NIOSH) indicates that hippuric acid in urine is stable at 4°C for 1 wk\textsuperscript{11}). Therefore, we assumed that the urinary HA concentration did not change during preservation.

Urinary creatinine was measured by an enzymatic method in the same laboratory, following the procedure reported by Yasuhara \textit{et al.}\textsuperscript{12)}. In order to adjust urinary HA concentration, urinary HA divided by urinary creatinine was used as corrected HA.

\textit{Chemicals}

We purchased chlorogenic acid, quinic acid, HA, caffeic acid, benzoic acid, HPLC-grade acetonitrile, and formic acid from Wako Pure Chemicals (Osaka, Japan). Tap water was purified using Milli-Q Element A10 (Millipore Japan, Tokyo, Japan) and used as pure water.

\textit{HPLC-electrospray ionization-tandem mass spectrometry measurement}

Standard solutions of chlorogenic, caffeic, quinic, HA, and benzoic acids were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Alliance 2695-Quattro Micro API, Waters, Milford, MA, USA). The levels of chlorogenic, caffeic, and quinic acids, and the absence of benzoic acid in the coffee were also analyzed using the same device.

A highly hydrophobic C30 stationary column (Develosil C30-UG-3 column; 150 mm long × 2.0 mm i.d., Nomura Chemical Corporation, Aichi, Japan) was used to analyze 500-µg/l standard solution and ten-fold
diluted coffee. The mobile phase was a linear gradient of solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). The program for solvent B was as follows: 0–8 min, 2→50%; 8–8.5 min, 50→100%; 8.5–9 min, held at 100%; 9–9.5 min, 100→2%; 9.5–20 min, held at 2%. The flow rate was 0.2 ml/min and the injection volume was 5 µl.

The coffee components were detected by positive electrospray ionization (ESI+): capillary voltage, 1 kV; ion source temperature, 120°C; desolvation gas temperature, 350°C; desolvation gas flow rate, 600 l/h; and cone gas flow rate, 50 l/h.

Multiple reaction monitoring (MRM) was used to confirm the detected substances. The MRM parameters used in these analyses are shown in Table 2.

This method employed the same device we used to determine N-methyl-2-pyrrolidone metabolites in urine as reported previously.\(^{13}\)

**Table 2. Parameters of multiple reaction monitoring**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Cone voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>355.1</td>
<td>163.0</td>
<td>20</td>
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</tr>
<tr>
<td>Quinic acid</td>
<td>193.1</td>
<td>147.0</td>
<td>20</td>
<td>10</td>
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<tr>
<td>Hippuric acid</td>
<td>180.1</td>
<td>76.9</td>
<td>20</td>
<td>32</td>
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<td>Caffeic acid</td>
<td>181.1</td>
<td>88.9</td>
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<tr>
<td>Benzoic acid</td>
<td>123.0</td>
<td>78.8</td>
<td>25</td>
<td>18</td>
</tr>
</tbody>
</table>

**Statistical methods**

Statistical analyses were performed using StatView 5.0 (SAS Institute Inc., Cary, NC, USA). Spearman’s rank correlation test was used to evaluate the relationship between urinary HA concentration and the amount of coffee consumed.

**Results**

**HA concentration**

Uncorrected urinary HA concentrations and urinary HA concentrations corrected for urinary creatinine concentration are summarized in Table 3.

Both uncorrected and corrected urinary HA concentrations increased with increasing coffee consumption (Figs. 1 and 2), and Spearman’s rank correlation test indicated a significant relationship between the HA concentration and the amount of coffee consumed (uncorrected, \(p<0.05\); corrected, \(p<0.01\)).

**Table 3. Urinary hippuric acid concentration after coffee intake**

<table>
<thead>
<tr>
<th>Case</th>
<th>HA (g/l)</th>
<th>Cre (g/l)</th>
<th>Corrected HA (g/g Cre)</th>
<th>HA (g/l)</th>
<th>Cre (g/l)</th>
<th>Corrected HA (g/g Cre)</th>
<th>HA (g/l)</th>
<th>Cre (g/l)</th>
<th>Corrected HA (g/g Cre)</th>
<th>HA (g/l)</th>
<th>Cre (g/l)</th>
<th>Corrected HA (g/g Cre)</th>
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<td>1</td>
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<td>1.12</td>
<td>0.11</td>
<td>0.14</td>
<td>1.23</td>
<td>0.11</td>
<td>0.27</td>
<td>1.12</td>
<td>0.24</td>
<td>0.25</td>
<td>0.62</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>0.26</td>
<td>1.19</td>
<td>0.22</td>
<td>0.12</td>
<td>0.97</td>
<td>0.12</td>
<td>0.21</td>
<td>0.48</td>
<td>0.44</td>
<td>0.19</td>
<td>0.38</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.18</td>
<td>0.94</td>
<td>0.19</td>
<td>0.12</td>
<td>1.22</td>
<td>0.1</td>
<td>0.37</td>
<td>0.85</td>
<td>0.43</td>
<td>0.53</td>
<td>1.16</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
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<td>0.6</td>
<td>0.05</td>
<td>0.07</td>
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<td>1.43</td>
<td>1.86</td>
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<tr>
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<td>0.14</td>
<td>0.01</td>
<td>0.49</td>
<td>0.01</td>
<td>0.12</td>
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<td>0.09</td>
<td>0.23</td>
<td>0.58</td>
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<tr>
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<td>0.04</td>
<td>0.01</td>
<td>0.55</td>
<td>0.02</td>
<td>0.01</td>
<td>0.51</td>
<td>0.09</td>
<td>0.03</td>
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</tr>
<tr>
<td>7</td>
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<td>1.03</td>
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<td>0.17</td>
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<td>0.22</td>
<td>0.6</td>
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<td>0.66</td>
<td>1.23</td>
<td>0.54</td>
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<tr>
<td>8</td>
<td>0.06</td>
<td>1.8</td>
<td>0.03</td>
<td>0.07</td>
<td>1.52</td>
<td>0.05</td>
<td>0.06</td>
<td>1.01</td>
<td>0.06</td>
<td>0.08</td>
<td>1.37</td>
<td>0.06</td>
</tr>
<tr>
<td>9</td>
<td>0.02</td>
<td>1.24</td>
<td>0.02</td>
<td>0.13</td>
<td>0.89</td>
<td>0.15</td>
<td>0.06</td>
<td>0.96</td>
<td>0.06</td>
<td>0.09</td>
<td>1.09</td>
<td>0.08</td>
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<tr>
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<td>1.28</td>
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<td>0.23</td>
<td>1.09</td>
<td>0.21</td>
<td>0.31</td>
<td>1.09</td>
<td>0.29</td>
<td>0.18</td>
<td>0.79</td>
<td>0.23</td>
</tr>
<tr>
<td>11</td>
<td>0.1</td>
<td>1.77</td>
<td>0.06</td>
<td>0.14</td>
<td>1.45</td>
<td>0.1</td>
<td>0.2</td>
<td>2.22</td>
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<td>0.23</td>
<td>1.11</td>
<td>0.21</td>
</tr>
<tr>
<td>12</td>
<td>0.08</td>
<td>0.94</td>
<td>0.09</td>
<td>0.08</td>
<td>1.62</td>
<td>0.05</td>
<td>0.38</td>
<td>1.15</td>
<td>0.33</td>
<td>1.28</td>
<td>2.89</td>
<td>0.44</td>
</tr>
<tr>
<td>13</td>
<td>0.04</td>
<td>0.5</td>
<td>0.08</td>
<td>0.21</td>
<td>1.07</td>
<td>0.2</td>
<td>0.08</td>
<td>0.71</td>
<td>0.21</td>
<td>0.62</td>
<td>1.89</td>
<td>0.33</td>
</tr>
<tr>
<td>14</td>
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<td>1.02</td>
<td>0.10</td>
<td>0.42</td>
<td>1.83</td>
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<td>0.09</td>
<td>0.36</td>
<td>0.25</td>
<td>0.14</td>
<td>0.57</td>
<td>0.25</td>
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<tr>
<td>15</td>
<td>0.03</td>
<td>0.66</td>
<td>0.05</td>
<td>0.41</td>
<td>1.17</td>
<td>0.35</td>
<td>0.35</td>
<td>1.16</td>
<td>0.3</td>
<td>0.11</td>
<td>0.25</td>
<td>0.44</td>
</tr>
<tr>
<td>Median</td>
<td>0.10</td>
<td>0.09</td>
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<td>0.23</td>
<td>0.25</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HA, hippuric acid; Cre, creatinine; Corrected HA, urinary HA adjusted by urinary creatinine.
HPLC-ESI-MS/MS measurements

MRM chromatograms of 5-µl aliquots of 500-µg/l standard solutions of chlorogenic acid, quinic acid, HA, caffeic acid, and benzoic acid are shown in Fig. 3, and those of a 5-µl aliquot of ten-fold diluted coffee are shown in Fig. 4.

The chromatograms indicated that the coffee consumed by the participants in this experiment did not contain benzoic acid, and that the concentrations of chlorogenic, quinic, and caffeic acids, and HA were 996.3 mg/l, 482.8 mg/l, 10.3 mg/l, and 2.2 mg/l, respectively.

Discussion

We allowed the participants to drink coffee at their convenient time during working hours because the resting times of and the ways adopted by workers who were coffee drinkers were generally different. Hence, we evaluated the influence of coffee intake on the urinary HA concentration under conditions similar to those observed in daily work. Moreover, we did not restrict the intake of any foods, except foods/drinks containing benzoic acid, because we sought to evaluate the influence of coffee intake on urinary HA concentration while maintaining the usual restrictions described in the regulations in Japan. In terms of the study design, our allowing the participants to take coffee at any time may be considered a limitation; our results may have been stronger had we performed a prior experiment in which the participants took coffee at a specified time prior to urine collection.
Our study showed that coffee intake influenced urinary HA concentration in toluene-nonexposed workers, even though the intake of food and drinks containing benzoic acid was controlled. It has been shown previously that green tea and/or coffee elevated the urinary HA levels in toluene-exposed workers. Taken together, these results indicate that the consumption of coffee does not accelerate toluene metabolism but rather that a component of coffee is itself metabolized to HA.

Spectrophotometric analysis revealed that chlorogenic and quinic acids were present at relatively high concentrations in coffee. These 2 components could be the main source of elevated HA levels in the body. Olthof et al. described a pathway in which chlorogenic acid is metabolized to HA as follows. Ingested chlorogenic acid reaches the colon and is hydrolyzed to caffeic and quinic acids by the colonic microflora. Subsequently, caffeic acid is dehydroxylated by the bacteria in the colon; after absorption, it is β-oxidized into benzoic acid in the liver. Quinic acid is dehydroxylated into cyclohexane carboxylic acid and aromatized into benzoic acid by the colonic microflora. Benzoic acid is conjugated with glycine and excreted in the urine as HA (Fig. 5). Our results show that urinary HA concentration in some participants was not elevated even after drinking 3 cups of coffee as compared to the HA concentration in those who did not drink coffee or had 1 or 2 cups of coffee. We suspect that this difference was mainly due to individual variations of microflora activity in the colon and the degree of absorption of the components into the circulation.

Our results showed that there was a significant relationship between HA concentration and coffee consumption in toluene-nonexposed workers; i.e., when a worker consumes coffee during working hours, he may have a high background level of HA that would influence the
toluene exposure estimate. Therefore, the toluene exposure of coffee-consuming workers may have been overestimated.

The chlorogenic acid content in the coffee used in this study was about 996 mg/l. Some studies have reported the chlorogenic acid contents in coffee. Olthof et al.\textsuperscript{6) reported} that 1 l of coffee contains 500–800 mg of chlorogenic acid. Monteiro et al.\textsuperscript{14) reported} a chlorogenic acid content of $1,068 \pm 49 \mu$mol/190 ml (1,991.6 ± 91.6 mg/l) (mean ± SD) in coffee. We analyzed the chlorogenic acid content in another coffee source, and found it to be 1,074 mg/l (chromatogram not shown). Thus, the coffee used in this study contained chlorogenic acid in the normal concentration range.

It has been reported that in humans, 75–100% of the ingested benzoic acid of up to 160 mg/kg body weight is metabolized to HA within 6 h, and the remaining is metabolized within 2–3 d\textsuperscript{15–17). We therefore suggest that as long as HA is used as a biomarker of toluene exposure, coffee intake should be restricted at least 2–3 d before biological monitoring in addition to the restriction on foods and drinks containing benzoic acid.

The measurement of urinary HA concentration is mandatory as per occupational health regulations in Japan; however, the occupational exposure limits set by the Japanese Society for Occupational Health and the biological tolerance values set by the Deutsche Forschungsgemeinschaft do not use urinary HA to monitor toluene exposure\textsuperscript{18, 19). Moreover, in 2009, ACGIH introduced a change in the biological monitoring of toluene and proposed that BEI should not include urinary HA\textsuperscript{20). It has been reported that urinary HA cannot be used to assess the exposure to low concentrations of toluene owing to the effect of its background concentration\textsuperscript{2). Therefore, it is recommended that the Japanese authorities reconsider the use of HA in the assessment of toluene exposure.

Fig. 4. Multiple reaction monitoring chromatograms of coffee. A 5 µl aliquot of ten-fold dilution of coffee was analyzed.

A: chlorogenic acid, B: quinic acid, C: hippuric acid, D: caffeic acid, E: benzoic acid.
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