Dietary Sources and Background Levels of Hippuric Acid in Urine: Comparison of Philippine and Japanese Levels

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Abstract: Levels of sodium benzoate in processed food from the Philippines and Japan were analyzed by high performance liquid chromatography. Results showed that of the 44 samples from the Philippines, 31 (70%) contained the compound. The samples with sodium benzoate included 19 juice, 6 softdrink and 6 soy sauce varieties. As for the Japanese products, only 8 (26%) out of 31 food items tested positive for sodium benzoate. The values of the compound in the Philippine samples ranged from 20 µg/ml to more than 2,000 µg/ml; the Japanese products showed a range of 50 to 200 µg/ml. Background urinary hippuric acid levels in 43 male Filipinos and 34 male Japanese with no occupational exposure to toluene were also measured using a high performance liquid chromatograph. Hippuric acid levels expressed as geometric means (SD) were 0.11 g/g creatinine (0.41) for the Filipino subjects and 0.09 g/g creatinine (0.39) for the Japanese subjects. No statistically significant difference in hippuric acid values in the 2 groups was noted. Possible explanations for the lack of any difference in background urinary hippuric acid levels between Filipino and Japanese subjects were discussed.

Key words: Sodium benzoate — Background hippuric acid — Filipino — Japanese

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INTRODUCTION

Hippuric acid in the urine is a biological marker for toluene exposure. However, hippuric acid may also be present in the urine of individuals who are not occupationally exposed to toluene. Consumption of certain food items containing benzoic acid or its compounds (occurring either naturally or added as antimicrobial) contributes to the background excretion of the metabolite.

A number of authors have estimated the level of background excretion. The ACGIH places this to be between 0.5–1.5 g/g creatinine. Differences in estimates may be due to differences in the amount of benzoic acid compounds in the diet.

The highest allowable amount of benzoic acid compounds that may be added to food is 0.1% in the Philippines and 0.6 g/kg (for softdrinks, syrups and soy sauce) in Japan. Judging from this difference, the ordinary Filipino diet may contain higher amount of benzoic acid compounds than the Japanese diet. This may result in higher background hippuric acid levels in Filipino urine than in Japanese urine.

Knowledge of the background excretion levels of hippuric acid is important particularly for countries reporting elevated metabolite levels among workers exposed to toluene. In 1991, a study in the Philippines showed that 78 out of 900 of workers exposed to organic solvent mixture had levels of urinary hippuric acid exceeding the BEI of 2.5 g/g creatinine. How much diet contributes to these levels is of considerable interest.

Thus, this study attempts 1) to determine the amount of benzoic acid or its compounds present in selected Philippine and Japanese processed food; 2) to measure urinary hippuric acid levels in Filipinos and Japanese who are not occupationally exposed to toluene; and 3) to compare the values in the 2 groups.

MATERIALS AND METHODS

Analysis of Food Items:

A total of 44 samples manufactured and consumed in the Philippines were analyzed. These samples included 6 soy sauce, 30 fruit juice, and 8 softdrink varieties. Similarly, 31 samples from Japan (5 soy sauce, 21 fruit juice and 5 softdrink) were tested. Samples were selected according to their availability on the shelves of popular chain food outlets in both countries. At least one product of each manufacturer available in a typical supermarket was collected for all the food types tested.

All food labels of the collected samples containing antimicrobial agents indicated the presence of sodium benzoate. For this reason, benzoic acid or its other compounds were measured as sodium benzoate.

Sodium benzoate levels in the samples were measured using a Shimadzu high performance liquid chromatograph (SCL-6B system controller, SIL-6B auto-injec-
tor) with a UV spectrophotometric detector (SPD-6AV) measuring absorbance at 225 nm. Quantitative analysis was done using the Shimadzu C-R4A data processor. Sodium benzoate in deionized water at concentrations between 10–100 μg/ml were used as standard solutions. The detection limit for sodium benzoate is 0.5 μg/ml. Operational conditions were as follows:

- **Column:** Shim-pack CLC-ODS 6.0 mmφ x 15 cm. (Shimadzu)
- **Guard column:** Shim-pack GRD-ODS 4.0 mmφ x 1 cm. (Shimadzu)
- **Mobile phase:** mixed solution of (1/30)M KH₂PO₄ and (1/30)M Na₂HPO₄ (2:1) with acetonitrile (20:1) at pH6.7
- **Flow rate:** 1.5 ml/min.
- **Pump pressure:** 80 kgf/cm²
- **Column temperature:** 40°C
- **Volume of sample injected:** 20 μl

One-ml samples were diluted 10 times using the mobile phase as the diluent. Juice concentrates were initially diluted with deionized water to a concentration suitable for drinking as directed by the manufacturers before final dilution with the mobile phase.

Before injection, all samples were filtered using a 0.45-μm membrane (Chromatodisc® 13A for HPLC, Kurabo, Japan). Samples that could not be filtered directly using the 0.45-μm filter were pre-filtered with 0.8-μm filters (Millex®-PF, Millipore Products Division, Massachusetts, USA).

**Analysis of Urinary Hippuric Acid:**

Thirty-four Japanese and 43 Filipino male subjects not occupationally exposed to toluene were included in the study. Urine samples from the Filipino subjects were collected 3-7 days before analysis. The samples were filtered in the Philippines with a 0.45-μm membrane (Chromatodisc® 13A for HPLC, Kurabo, Japan) and stored in 1.5-ml polypropylene tubes at a temperature below 0°C soon after filtration. The samples were then transported for analysis to Japan inside an insulated vessel containing a coolant. The temperature was kept below 4°C. Urine samples taken from the Japanese subjects were prepared as described above and analyzed on the same day of collection.

Before the above samples were collected and analyzed, the stability of hippuric acid and creatinine was checked in the prefiltered urine stored at room temperature (average: 26°C) and at 4°C. No significant change in either of these parameters was noted up to 7 days for urine stored at room temperature and up to 12 days for urine stored at 4°C (results not shown).

Creatinine and hippuric acid in the samples were simultaneously determined using the method suggested by Takeuchi Y. et al. for HPLC analysis. The calibration curve was made using standard solutions in the mobile phase having concentrations between 50 μg/ml and 150 μg/ml for creatinine and between 50 μg/ml and 250 μg/ml for hippuric acid. The detection limit for hippuric acid is 1
μg/ml. Operational conditions were the same as for sodium benzoate analysis except for the following items:

- UV spectrophotometer absorbance: 227 nm
- Mobile phase: Deionized water (800): Acetonitrile (200): Acetic acid (15) (in volume) pH 2.7
- Flow rate: 1.2 ml/min.
- Volume of sample injected: 10 μl

Before injection for analysis, the urine samples were diluted 20 times with the mobile phase. Hippuric acid levels were corrected for urinary creatinine excretion.

Reagents Used:
All chemicals used were of reagent grade and were supplied by Wako Pure Chemical Industries Ltd., Japan.

Statistical Analysis:
Student’s t-test for 2 independent samples was conducted to determine statistically significant difference in the hippuric acid levels of Filipinos and Japanese.

RESULTS

A. Sodium Benzoate Analysis

About 70% of the food items tested from the Philippines were positive for sodium benzoate (Table 1). In contrast, only 8 Japanese products were found to contain the compound. Important to note also is the high percentage (63%) of Philippine-manufactured fruit juices containing sodium benzoate (vs. Japan: none). All softdrinks with sodium benzoate in Japan are the diet-type. In the Philippines, however, both diet (4 out of the 8 tested) and regular softdrinks (2 out of 8 tested)

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>Sodium Benzoate Content</th>
<th>TYPE OF FOOD ITEM</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Juice</td>
<td>Soft drink</td>
</tr>
<tr>
<td>Philippines</td>
<td>No. of Items Tested</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>No. of Items (+) for Sodium Benzoate</td>
<td>19 (63%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Japan</td>
<td>No. of Items Tested</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No. of Items (+) for Sodium Benzoate</td>
<td>0 (0%)</td>
<td>3 (60%)</td>
</tr>
</tbody>
</table>
contain the compound. Among the different types of food items analyzed, soy sauce gave the highest percentage (100%) for both countries.

Soy sauce from the Philippines showed the highest levels of the compound with a maximum of more than 2,000 μg/ml (Fig. 1). For fruit juice drinks containing sodium benzoate, the average was 310 μg/ml while the highest value was 800 μg/ml. Of the regular softdrinks, one contained 100 μg/ml and the other, about 700 μg/ml of the compound. Diet softdrinks had levels of sodium benzoate between 100-700 μg/ml.

In comparison, the level of sodium benzoate in the 8 Japanese products ranged from less than 50 μg/ml to 200 μg/ml. The 6 soy sauce varieties tested had levels of around 100 μg/ml or less (average = 86 μg/ml). The levels of sodium benzoate in the 3 Japanese diet softdrinks ranged between 150–200 μg/ml.
B. Urinary Hippuric Acid Analysis

Random urine was collected from 43 male Filipinos and 34 male Japanese for this study. The job descriptions of the study population are shown in Table 2. All the Japanese subjects work for the same health research institute. The Filipino subjects were selected purposively and work in different institutes and offices.

Hippuric acid levels were transformed into logarithmic values and were expressed as geometric mean and geometric standard deviation. Statistical analysis using t-test showed no significant difference in the values between the Filipino and Japanese groups (Table 3).

Figure 2 clearly shows the tendency and spread of the corrected urinary hippuric acid levels in the two populations. Despite the presence of one outlier among the Filipinos (hippuric acid: 0.8 g/g creatinine), about 75% of the subjects for both groups had hippuric acid levels with less than 0.2 g/g creatinine.

**DISCUSSION**

The maximum allowable level of sodium benzoate that may be added to food is 0.1% in the Philippines. This is almost twice that in Japan, where the permissible amount is 0.6 g/kg. This difference in policies is reflected by the higher levels of sodium benzoate in all food types tested from the Philippines as compared to items from Japan. This study also established that, in general, more food items from the Philippines contain sodium benzoate. Judging from these results, it may be inferred that Filipinos consume larger amounts of sodium benzoate than the Japanese. Moreover, the potential for consuming food products with sodium benzoate is seemingly further increased by the fact that Filipinos drink more cold beverages (such as softdrinks and fruit juices) because of the high year-round temperature in the Philippines.

Several authors have measured background hippuric acid levels for Japanese. In 1969, Ikeda et al. found the level to be 0.23 g/g creatinine for males and 0.45 for females. In 1972, Tomokuni et al. reported a mean of 0.44 g/l. No estimates of background levels have been made for Filipinos.

The mean background level of hippuric acid for Japanese subjects in this study

**Table 2. Job descriptions of study population from the Philippines and Japan.**

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>n</th>
<th>JOB DESCRIPTION (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FILIPINOS</td>
<td>43</td>
<td>Office Workers (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medical Workers (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others (2)</td>
</tr>
<tr>
<td>JAPANESE</td>
<td>34</td>
<td>Office Workers (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Researchers (25)</td>
</tr>
</tbody>
</table>
was 0.09 g/g creatinine. This is much lower than the previous estimates. Noting that the earlier measurements were done more than 20 years ago, advancement in food processing techniques excluding the use of benzoic acid and its compounds may explain the lower values found in this study.

The results of sodium benzoate levels in the food analyzed imply that Filipinos have higher background hippuric acid levels as compared to the Japanese. However, no statistically significant difference in urinary background levels was noted by the authors. It therefore seems plausible that several factors obscure the relationship between diet and metabolite levels.

One factor is that the two sample populations may not be comparable in terms

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>n</th>
<th>RANGE (g/g creatinine)</th>
<th>Geometric Mean (SD) (g/g creatinine)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>FILIPINOS</td>
<td>43</td>
<td>0.01–0.80</td>
<td>0.11 (0.41)</td>
<td>NS</td>
</tr>
<tr>
<td>JAPANESE</td>
<td>34</td>
<td>0.02–0.43</td>
<td>0.09 (0.39)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Urinary hippuric acid levels in Filipinos and Japanese occupationally unexposed to toluene.
of composition. As already mentioned, the Japanese subjects all came from the same institute while the Filipino subjects, on the other hand, came from different offices and institutes. The influence of socioeconomic factors in the choice of food to be consumed may have been overlooked because of homogeneity of either sample population.

Also, differences in the metabolism of ingested sodium benzoate may come into play when comparing groups of diverse ethnicity. This relationship can only be established by additional research.

The results, notwithstanding, should not obscure the significance of knowing background hippuric acid levels in the interpretation of biological exposure levels. Correction for background excretion may render urinary hippuric acid levels more useful in the assessment of toluene exposure. This is of considerable importance for countries where limit values are adapted from other countries. In this particular case, evaluation of metabolite levels in the Philippines is based on the ACGIH values.

ACKNOWLEDGMENTS

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