Urinary Hippuric Acid Excretion in Everyday Life

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The biological monitoring for exposure to toluene is based on the measurement of urinary hippuric acid concentration (HA-U). In occupational health, workers exposed to toluene are examined for HA-U, because collection of urine samples is easy.

The HA-U of some designers in a great household electrical products corporation who did not use toluene occupationally showed relatively high values. From statistical analyses, it was revealed that the HA-U values of the designers who lived without family members were higher than those with family members and that the values on Monday were higher than those on Friday. Some factors which elevate the HA-U level are considered in individuals living alone and/or in life at weekends. These may be to drink many soft drinks, especially low calorie ones, in which benzoic acid is used as a preservative.

(Key Words: urinary hippuric acid, benzoic acid, preservative, soft drinks, food survey, life without family members)

INTRODUCTION

Toluene is the most popular organic solvent in industry (6). Part of the absorbed toluene is eliminated in exhaled breath, but a large percentage is oxidized to benzoic acid, conjugated with glycine, and excreted as hippuric acid (HA) in the urine (3, 11) according to the chemical reaction as shown in Fig. 1. The biological monitoring for exposure to toluene is based on the measurement of urinary hippuric acid concentration (HA-U) (11, 12). In occupational health, workers exposed to toluene are examined for HA-U, because collection of urine samples is easy.

The HA-U of some designers in a great household electrical products corporation who designed new planned appliances and did not use toluene occupationally showed relatively high values. The reasons why the HA-U values were high were discussed.

MATERIALS AND METHODS

1) Subjects
The subjects were seventy designers (male:64, female:6) of a great household electrical products corporation in Tokyo. Their work involves inventing and drawing designs for each new planned appliance. The ages of the male and the female designers were 22–55 and 24–30 years with mean values of 34.1 and 26.5 and S.D.s 8.6 and 2.3 years in Sep. 1987 respectively. They use many kinds of markers and one kind of gradation solvent and sometimes use other organic solvents.

2) Measurement of HA-U and urinary methylhippuric acid (MHA-U)
To measure levels of HA-U and MHA-U, high performance liquid chromatography was used.

3) Measurement of solvents in materials and occupational air samples

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The concentrations of organic solvents in materials and in air of occupational environment were measured by gas chromatography.

4) Questionnaire

Items of the questionnaire to investigate their private life were identification number, name, sex, age, private life status (life without or with family members, which does not mean marital status), private use of organic solvents, to take foods with preservatives (especially soft drinks), prunes [contain much benzoic acid (17)] and much protein (especially on weekend), exercise (especially on weekend), and household medicines (especially aspirin).

RESULTS

The forty designers who used sometimes organic solvents in the section of designers (Design Center) were examined for HA-U and MHA-U during a few days in July 1986, because they used many kinds of organic solvents. The MHA-U values were all 5 mg/l or less, and the seven HA-U values were 750 mg/l or more with maximum 2235 mg/l. Then, the HA-U of all the members in the Design Center who used organic solvents were measured in the morning (8:30–9:00) on Sep. 22 (Mon.) and in the afternoon (15:30–16:30) on Sep. 26 (Fri.), 1986. A few subjects whose urine samples were not collected on Sep. 22 or 26 were examined for HA-U on the next days (Sep. 23 or 29). The HA-U values of subjects whose HA-U values were 750 mg/l or more on Sep. 22 or 26 were measured in the afternoon on Oct. 24 (Fri.), 1986. The HA-U values on Oct. 24 were 46–619 mg/l. On the day in the autumn of 1986, concentrations of organic solvents in the air of the Design Center were measured and the concentrations of xylene were at most several ppm, whereas other organic solvents were not detected. Sixty-nine of the seventy designers were examined for HA-U during a few days in January 1987.

Table 1 shows the means, S.D.s, and numbers of samples with maxima and minima on Sep. 22 (Mon.) and 26 (Fri.) 1986, in January 1987, and for maximum of individual values, for both sexes and male. Paired t-tests between means on Sep. 22 (Mon.) and 26 (Fri.) are also shown in Table 1. This table indicates that the HA-U level on Monday is significantly higher than that on Friday.

Some factors which elevate the HA-U levels are considered in private life, then the private lives of the seventy designers were investigated by the above questionnaire from August to September 1987. Means, standard deviations, and numbers of samples by factors, which were (i) sex and (ii) life without or with family members, and the Welch's t-tests for differences between the means by the factors were calculated, by using the maxima of the individual HA-U values. Table 2 shows the above results. Table 2 indicates remarkable statistical significance for differences between the maximum HA-U values for private life status without and with family members and between the values of both sexes, i.e. the HA-U value for private life status without family members or for female is significantly higher than that for private life with family members or for male. Statistical differences among or between the HA-U values by other factors than sex, private life status, and data in the questionnaire to investigate private life, however, could not be found. Age was not found to be confounding factor which affected the HA-U level in this study. The same trends were detected by statistical analyses for discrete
Table 1  Means, standard deviations, numbers of samples, maxima, and minima of the HA-U for the designers by date and paired t-tests between means on Monday and Friday

<table>
<thead>
<tr>
<th></th>
<th>both sexes</th>
<th></th>
<th></th>
<th></th>
<th>male</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>S.D.</td>
<td>No.</td>
<td>maximum</td>
<td>minimum</td>
<td>mean</td>
<td>S.D.</td>
<td>No.</td>
<td>maximum</td>
<td>minimum</td>
</tr>
<tr>
<td>Sep. 22, 1986 (Mon.)</td>
<td>486mg/l</td>
<td>517mg/l</td>
<td>69</td>
<td>3490mg/l</td>
<td>17mg/l</td>
<td>420mg/l</td>
<td>368mg/l</td>
<td>63</td>
<td>1540mg/l</td>
<td>17mg/l</td>
</tr>
<tr>
<td>Sep. 26, 1986 (Fri.)</td>
<td>293mg/l</td>
<td>173mg/l</td>
<td>66</td>
<td>1223mg/l</td>
<td>36mg/l</td>
<td>295mg/l</td>
<td>278mg/l</td>
<td>60</td>
<td>1223mg/l</td>
<td>36mg/l</td>
</tr>
<tr>
<td>Jan. 1987</td>
<td>429mg/l</td>
<td>485mg/l</td>
<td>69</td>
<td>2577mg/l</td>
<td>5mg/l</td>
<td>358mg/l</td>
<td>376mg/l</td>
<td>63</td>
<td>1715mg/l</td>
<td>5mg/l</td>
</tr>
<tr>
<td>individual maximum</td>
<td>672mg/l</td>
<td>603mg/l</td>
<td>70</td>
<td>3490mg/l</td>
<td>56mg/l</td>
<td>575mg/l</td>
<td>427mg/l</td>
<td>64</td>
<td>1715mg/l</td>
<td>56mg/l</td>
</tr>
<tr>
<td>paired t-test between</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep. 22 and 26, 1986</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.91(64)**</td>
<td></td>
</tr>
<tr>
<td>t(df)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.48(58)*</td>
<td></td>
</tr>
</tbody>
</table>

remarks: **: p<0.01, *: p<0.05

Table 2  Means, standard deviations, and numbers of samples of the HA-U by sex and private life status and Welch's t-tests for differences between the means by the factors

<table>
<thead>
<tr>
<th>private life status</th>
<th>male mean</th>
<th>S.D.</th>
<th>No.</th>
<th>female mean</th>
<th>S.D.</th>
<th>No.</th>
<th>total mean</th>
<th>S.D.</th>
<th>No.</th>
<th>Welch's t-test t(df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>without family members</td>
<td>776mg/l</td>
<td>469mg/l</td>
<td>22</td>
<td>1910mg/l</td>
<td>1350mg/l</td>
<td>4</td>
<td>951mg/l</td>
<td>760mg/l</td>
<td>26</td>
<td>3.23(3.1)*</td>
</tr>
<tr>
<td>with family members</td>
<td>470mg/l</td>
<td>367mg/l</td>
<td>42</td>
<td>1300mg/l</td>
<td>771mg/l</td>
<td>2</td>
<td>507mg/l</td>
<td>416mg/l</td>
<td>44</td>
<td>3.00(1.0)</td>
</tr>
<tr>
<td>total</td>
<td>575mg/l</td>
<td>427mg/l</td>
<td>64</td>
<td>1710mg/l</td>
<td>1140mg/l</td>
<td>6</td>
<td>672mg/l</td>
<td>603mg/l</td>
<td>70</td>
<td>5.12(5.1)**</td>
</tr>
<tr>
<td>Welch's t-test t(df)</td>
<td>2.88(34.8)**</td>
<td></td>
<td></td>
<td>0.57(3.6)</td>
<td></td>
<td></td>
<td>3.16(34.0)**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

remarks: **: p<0.01, *: p<0.05
data which were odds ratios and chi-square tests by the Mantel-Haenszel's methods (9, 4, 2). Continuous data of the HA-U were changed into the dichotomous data at cut-off point 750 mg/l. The upper limit of the so-called normal HA-U has been reported to be 500–1000 mg/l or at least 1000 mg/l in Japan (10, 11) for the biological monitoring of toluene.

The dealers of the materials in the Design Center reported that the main ingredients of the markers were xylene ($o$-, $m$-, and $p$-) and butylalcohols ($n$-, and iso-) and that those of the gradation solvent were trimethylbenzenes ($1$, $2$, $3$-, $1$, $2$, $4$-, and $1$, $3$, $5$-). These chemicals were confirmed by gas chromatography using the standard organic solvents at the Department of Occupational Health and Industrial Hygiene, School of Medicine, Tokai University in 1986. The dealers have not shown that toluene is one of the main ingredients of the materials.

**DISCUSSION**

The HA-U and the MHA-U values of the designers were measured in July 1986, because they used sometimes many kinds of organic solvents and at that time the Medical Service Center of the corporation did not know that they did not use toluene occupationally.

The biological monitoring for exposure to toluene is based on the measurement of HA-U (12). In occupational health, workers exposed to toluene are examined for HA-U, because collection of urine samples is easy. High HA-U values of the designers who did not use toluene occupationally did not consist with the biological monitoring system in occupational health.

In order to detect the reason why the HA-U values of the designers who did not use toluene occupationally were high, the private lives of these designers were investigated by using the above questionnaire.

Sugita et al. (14) reported that the HA-U levels of an engineer who did not use toluene occupationally were elevated by toluene used privately, whereas it was revealed that the designers seldom used toluene privately in this article.

Remarkable findings detected for the HA-U levels of the designers were (i) Monday > Friday, (ii) female > male, and (iii) life without family members > life with family members. Number of the female designers examined was six. Therefore, meaning of the difference between the levels by sex is not significant. Higher HA-U level on Monday than that on Friday indicates that some factors in private life on weekend elevate their HA-U values, and occupational exposure to toluene does not affect them. The reason why the HA-U level of the designers without family members was higher than that with family members is a notable problem.

It is well known that the biological monitoring for the exposure to toluene using HA-U is of low specificity, i.e. high HA-U is sometimes detected without exposure to toluene. Therefore, in occupational health, the biological monitoring by using HA-U should be the group monitoring that HA-U means among several groups exposed to different toluene levels are compared with one another (12). As shown in Fig. 1, benzoic acid (BA) is metabolized to HA by being conjugated with glycine ($8$, $17$) in liver. Therefore, high HA-U is sometimes caused by intake of BA (15). BA is naturally present in many fruits and vegetables, especially cranberries, prunes, and coffee beans (13, $17$). As a preservative for foods, in Japan, BA is added to foods, especially soft drinks, soy sauce, syrup, and caviar (16). The maximum limits of BA contained in soft drinks, soy sauce, and syrup are 0.6 g/kg (16). Mean BA concentration in soft drinks (including fruit juices) was 0.085 g/kg ($n = 797$) with maximum 0.570 g/kg, and 51.2% of soft drink samples contained BA in Japan (16). If a mammal is given water whose BA concentration is 0.570 g/kg and if all of the BA is metabolized to HA in liver, elevation of the HA-U level is theoretically $+836$ mg/l from calculating their molecular weights.

From the above results, it is reasonable to assume that some factors in private life without family members, especially on weekends, must elevate the HA-U level. From information obtained by the questionnaire, neither increased intake of soft drinks nor other private life style related to the elevation of the HA-U level. Therefore, the factors which elevate the HA-U level may not include drinking many soft drinks with BA in private life. Food surveying using either a questionnaire, an interview, or a total diet collection method would unavoidably involve errors because of the variety of individu-
al eating habits. Therefore, it may be reasonable that a statistical relationship between the elevation of the HA-U level and response to the questionnaire for large soft drink intake was not detected, if one of the factors which elevate the HA-U level is taking many soft drinks with BA. It may be mentioned that persons who live without family members consume more soft drinks and less green tea (which does contain only a little amount of BA) than those with family members in Japan, because making green tea is troublesome for those without family members. Cans and bottles of some of low calorie soft drinks (especially cola) have containing BA as a preservative printed on them. Many women must like low calorie soft drinks considering diet, which may not be discrepant with the HA-U level of the female designers being higher than that of the male designers. Two male designers, whose HA-U values were 818 mg/l and 1373 mg/l on Sep. 22, 1986 and 1025 mg/l and 78 mg/l on Sep. 26, had lived without family members, and they married from October to December 1986. After marriage their HA-U values were 154 mg/l and 46 mg/l in January 1987, which expresses remarkably influence of the private life status on the HA-U level.

In analytical epidemiology, reasonable and significant relationships between mortality or morbidity rates of adult diseases (i.e. cancers, coronary heart disease, cerebrovascular diseases, and other) and results in food surveys have rarely been detected in Japan recently (7, 18). Then, the food survey with too many and large errors involved in the results is sometimes criticized (1, 5). Because variety of eating habits is notable, i.e. food which an identical individual eats varies remarkable everyday, whereas to live without or with family members (private life status) is reliable information. Therefore, it is appropriate that a significant relationship between the HA-U level and private life status was detected, while that between the HA-U level and response to the questionnaire to drink many soft drinks was not detected. It is mentioned that taking many soft drinks is not similar to response to the questionnaire to take many soft drinks.

The group of designers is a special one, because their job is art. Therefore, these results cannot be generalized.

Concentration or dilution of urine affects HA-U, therefore, concentrations of chemicals in urine should be corrected with gravity of urine (GU) or urinary creatinine concentration (8, 11). In this study, the GU of the designers was not examined because of restriction to measure samples in occupational fields. Errors affected by urine concentration or dilution can be ignored by the measurement of spot samples for the seventy designers.

As shown in Fig. 1, phenylalanine and glycine are considered as other factors which affect HA-U (17). Phenylalanine and glycine are amino acids of which proteins consist. It is thought that the intake of a large amount of protein and/or accelerated catabolism of protein may be the causes of HA-U elevation. When a person takes much exercise, in general, his or her amount of protein intake increases. Significant relationships between these two factors and the HA-U level were not detected in this study, therefore, they should be studied in more detail later.

We must consider whether taking aspirin can elevate the HA-U level or not, because the chemical structures of aspirin and HA are similar. In this study, no designers took aspirin usually or occasionally, therefore this relationship could not be detected. It must be studied later.

REFERENCES


190—M. SUGITA et al.