Possible Effects of Drinking and Smoking Habits on Hippuric Acid Levels in Urine of Adults with No Occupational Toluene Exposure

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Abstract: Drinking and Smoking Habits and Urinary Level of Hippuric Acid in Adults not Exposed to Toluene: Edna Maria Alvarez-Leite, et al. Department of Clinical Chemistry and Toxicology, College of Pharmacy, Federal University of Minas Gerais-UFMG, Brazil—Hippuric acid (HA) is still the biomarker most used for monitoring exposure to toluene, but it is produced by the body even in the absence of this solvent, and has the disadvantage of showing significant variation in and between individuals, depending on environmental factors and individual characteristics. A number of studies have reported the influence of individual drinking and smoking habits on toluene metabolism, but the effect on urinary excretion of HA is still controversial. This study was conducted in an attempt to examine whether these individual habits also affect HA excretion in individuals not exposed to toluene. Urine sample from 195 people (99 women and 96 men), ranging in age from 17 to 46 years old, were collected. The individuals were classified in groups according their drinking and smoking habits. The data from the current study indicate that these two social habits, either separately or combined, do not influence basal urinary HA levels in this study group.

Key words: Urinary hippuric acid, Smoking habits, Drinking habits

The peripheral biomarker of exposure depends, amongst other things, on the basal values for individuals who have not been exposed. With the progressive improvement in working environments, at least in the majority of countries, levels of biological markers are becoming closer to their background levels, a fact which emphasises the pressing need for an understanding of their base levels and the factors which influence them.

Hippuric acid (HA) is still the biomarker most used for monitoring exposure to toluene, but it is produced by the body even in the absence of this solvent, and has the disadvantage of showing significant variation in and between individuals, depending on environmental factors and individual characteristics.

A number of studies have shown that toluene metabolism can vary as a function of such as genetic polymorphism, diet, alcohol consumption, cigarette-smoking, as well as body surface. The effect of ethanol, especially, has been the subject of numerous studies. It is known that alcohol interacts with toluene metabolism, and has both stimulating (chronic consumption) and inhibiting (acute consumption) effects on the solvent metabolism. This is a possible explanation for the controversial effects of alcohol consumption on urinary excretion of HA in humans exposed to toluene, reported by different studies. Dossing et al. recorded a reduction, whereas Bavazzano et al. recorded an increase, in the elimination of hippuric acid into urine as a result of alcohol consumption. Hjelm et al. found that the excretion of this metabolite is reduced when alcoholic drinks are combined with a carbohydrate restricted diet.

Another factor which plays an important role in toluene metabolism is cigarette-smoking. According to Inoue et al., the combination of alcohol and cigarettes reduces the HA in the urine of workers exposed to toluene, and this association would be a confounding factor in the separate evaluation of the effects of the two habits on toluene metabolism.

These studies indicate the need for a careful evaluation of the effects of smoking and alcohol consumption when monitoring occupational exposure to toluene with urinary HA as a biomarker. The important question addressed here is whether these habits affect HA excretion into urine.
from non-exposed individuals i.e. basal HA excretion, increasing as such individual variation in the levels recorded.

Materials and Methods

Sampling Procedure

Urine samples were obtained from 195 people (99 women and 96 men), ranging in age from 17 to 46 years old, who had not been exposed to toluene. They were all residents of the metropolitan region of the city of Belo Horizonte, Minas Gerais, Brazil. The selected individuals were asked to reply to a toxicological protocol at the time when the samples were taken. There were five exclusion factors: renal, hepatic or pulmonary illness of any sort, use of medicines, and the ingestion of preserved foods involving the use of benzoic acid or benzoate within the previous 24 h. The individuals were divided into the following groups: control group (C) - did not smoke or drink alcohol (n=92); experimental group 1 (E1) - drank alcohol but did not smoke (n=44); experimental group 2 (E2) - smoked cigarettes but did not drink alcohol (n=30); experimental group 3 (E3) - smoked cigarettes and drank alcohol (n=29).

The individuals comprising E2 and E3 smoked between 5 and 30 cigarettes a day, with a mean of 12. The blood alcohol level was not determined in the members of groups E1 and E2, but all of them were occasional alcohol drinkers and they had drunk alcoholic beverage at least once during the 24 h before the sample collection.

Statistical analysis

The statistical analysis of the results was carried out with the Jandel SigmaStat® package for Microsoft Windows®. The Kolmogorov-Smirnov test was used to check for normal distribution of the results. The Mann-Whitney Rank Sum Test was used to compare mean values for hippuric acid in the control group and the experimental groups. The effect of gender, age and number of cigarettes smoked a day on HA excretion were examined by multiple linear regression on the log values of HA (gender scored: 0- male, 1- female).

Hippuric acid analysis

Hippuric acid was determined in urine samples by gas chromatography by the method of Kira, modified by Alvarez-Leite et al. After methylation with trimethylphenylammonium hydroxide the samples were analysed by gas-chromatography with an ionizing flame detector (FID) (Instrumentos Científicos CG LTD, Brazil).

Results

The GC method used in this work has resulted in a detection limit of 0.10 g/l, CV (intra and inter assay) of 8 and 14% respectively and a rate of recovery from spiked urine sample of 89.1%. The urinary hippuric acid level in the total group of samples (n=195) varied from <0.10 to 1.61 g/l (mean 0.37 g/l).

Table 1 shows the means, median and minimum and maximum values found for urinary hippuric acid in the control and experimental groups 1, 2 and 3. HA values showed a non normal distribution and log transformed values were used to study the effect of gender and age on metabolite urinary excretion. The urinary HA level was not significantly influenced by gender or age (p>0.05) in either control or experimental groups.

No significant difference was found between urinary HA values in groups C and E1 (p>0.05) and C and E2 (p>0.05). The mean hippuric level of E3 (smokers who drank alcohol) was the lowest (0.34 g/l) in the four groups analysed, but was still not significantly different from the control group (p>0.05), from the experimental group 1 (non-smokers who drank alcohol) (p>0.05) nor from the experimental group 2 (smokers but did not drink alcohol) (p>0.05). Multiple linear regression did not show any significant correlation between HA levels and the number of cigarettes smoked a day either in group E2 or E3.

Table 1. Urinary hippuric acid in the control group and experimental groups

<table>
<thead>
<tr>
<th>Hippuric acid (g/l)</th>
<th>Groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>92 (54 F–38 M)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>33.0 ± 13</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.37</td>
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<tr>
<td><strong>Median</strong></td>
<td>0.28</td>
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<tr>
<td><strong>Min</strong></td>
<td>&lt;0.10</td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td>1.61</td>
</tr>
</tbody>
</table>

F=female, M=male. E1=drink alcohol but do not smoke, E2=smoke but do not drink alcohol, E3=drink alcohol and smoke. 1 The limit of quantification was 0.10 g/l. 2 Lowest concentration of HA recorded in the group. 3 Highest concentration of HA recorded in the group.

Discussion

Taking into account that the basal level of HA plays an important role in the interpretation of the results of biological monitoring of exposure to toluene, we attempted to evaluate if the effects of individual smoking and drinking habits affect the urinary excretion of HA in adults not exposed to solvent. Both positive and negative effects of drinking alcohol or cigarette smoking on urinary
HA excretion have been recorded by various authors, but in almost all cases they were particularly examining the effects of tobacco and alcohol on toluene metabolism not on background HA levels. The comparisons between the control group and the experimental groups were made independent of gender and age, as these had previously been shown to have no effect on basal urinary HA levels.

The data from the current study indicate that the two social habits of drinking and smoking do not, independently, influence basal urinary HA levels. The mean urinary HA level in group E, (drinkers but not smokers) was the highest among the groups analysed, but it was not significantly different from that of the control group. It was also found that there was no significant correlation between urinary HA and the habit of smoking (control group and experimental group 2) even when the number of cigarettes smoked a day was considered. These results agree with the findings of Bazzano et al. who also reported no effect of drinking or smoking on the basal level of urinary HA.

Inoue et al., studying workers exposed to toluene, found a marked reduction in HA elimination in those who habitually smoked and drank alcohol. The same result was not found among individuals not exposed to toluene, as reported here. Although the mean hippuric level of E3, (drinkers and smokers) was the lowest (0.34 g/l) among the four groups analysed, there was no significant difference in urinary HA levels from those of the control group.

In conclusion, the results presented here showed that the individual habits of smoking and drinking, either separately or combined, did not significantly alter urinary HA levels. The comparisons between the control group and experimental groups were made independent of gender and age, as these had previously been shown to have no effect on basal urinary HA levels.

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