Research Note:
Urine Trans,trans-muconic Acid as a Biomarker for Benzene Exposure in Gas Station Attendants in Bangkok, Thailand

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Abstract. The toxicity of benzene, a chemical used in many industrial processes, involves bone marrow depression and leukemogenesis and is associated with damage to multiple classes of hematopoietic cells and hematopoietic functions. Environmental exposure to benzene causes an increased body burden, which is reflected in several biomarkers, eg, urine trans,trans-muconic acid (ttMA). Associated with the industrialization of Thailand, a developing country in Southeast Asia, workers in many occupations have acquired substantial risks of benzene exposure. In this study, benzene exposure was monitored by high-performance liquid chromatography (HPLC) of urine ttMA in 79 persons, including 49 controls and 30 gas station attendants. In controls, urine ttMA concentration averaged 0.12 (SD ± 0.03) mg/g creatinine; in gas station attendants, urine ttMA concentration averaged 4.00 (SD ± 12.49) mg/g creatinine (p < 0.05). Based on these findings, wider use of urine ttMA determination is recommended as a biomarker for occupational exposure to benzene. (received 31 July 2001; accepted 5 August 2001)

Keywords: benzene, gas station attendants; occupational and environmental health, biomarker

Introduction

Benzene is an important chemical agent that is used in many industrial processes. Its toxicity involves bone marrow depression and leukemogenesis caused by damage to multiple classes of hematopoietic cells and various hematopoietic cell functions [1]. Potential mechanisms whereby benzene can damage cellular macromolecules include (a) covalent binding of reactive metabolites, and (b) the capacity of benzene metabolites to induce oxidative damage [2].

Environmental or occupational exposure to benzene results in increased body burden, which is reflected in several biomarkers of benzene exposure. These include the urinary levels of benzene and its metabolites, as well as adducts of reactive benzene metabolites with cellular constituents. Assays of such biomarkers can be used to identify populations with significant exposures to benzene, to estimate benzene exposure levels, and to delineate differences in benzene metabolism [3,4].

Trans,trans-muconic acid (ttMA) is a minor non-phenolic metabolite of benzene that is excreted in urine. Assay of urine ttMA has been recommended as a biomarker for benzene exposure [5-7]. The urine metabolites that might serve as indices of occupational or environmental exposures to benzene include phenol, hydroquinone, ttMA, and S-phenylmercapturic acid. Of these, urine ttMA concentration is increasingly recognized as a reliable biomarker that is relatively convenient to measure [8,9].

Due to the recent industrialization of Thailand, a developing country in Southeast Asia, workers in many occupations appear to be at high risk for benzene exposure. We previously provided the initial evidence that Thai mechanics have high exposure to benzene, based on monitoring their urine ttMA concentrations [10]. Among the other occupations with high risk for benzene exposure are gas station attendants, who are the focus of this investigation.
During their daily work, gas station attendants have direct contact with petroleum products, so occupational exposure to benzene cannot be avoided. In Thailand, most gas station attendants come from rural areas where they receive scanty education about personal health-care. The present report describes a pilot study to compare urine tMA concentrations in a group of gas station attendants versus those in a comparable group of non-exposed control subjects.

Materials and Methods

Subjects. The study group included 30 male gas station attendants who worked full-time at gas stations in the Ladprao district of Bangkok, Thailand. All of the workers were non-smokers in compliance with the strict “no-smoking” rules of gas stations. The stations were located in the business area of Bangkok, where the monitored level of atmospheric benzene is 0.76 - 4.14 ppm [11].

The control group included 49 healthy men with low risk for benzene exposure. All were villagers from rural areas without nearby factories. All were non-smokers. These subjects were selected as a reference group for our laboratory and they were included in our previous study [10].

All of the 79 subjects provided informed consent. A random urine sample was collected from each subject on a workday between 2 pm and 7 pm, about 4 to 8 hr after the subjects started to work.

Assay for tMA in urine. Assays of tMA concentrations in the urine samples were performed by HPLC, as previously described [12]. Briefly, 0.5 ml of urine was mixed with 2 ml of tris-HCl buffer (pH 10) containing vanillic acid as an internal standard. This mixture was percolated through a preconditioned ion-exchange column (Dowex I, 100-200 mesh, 1 cm diameter, 10 cm height). After the column was rinsed with phosphoric acid solution, acetate buffer, and deionized water, the analyte was eluted in 2 ml of a solution comprising equal volumes of 1.5 M sodium chloride solution and methanol. Of this, 10 µl was injected onto a HPLC column (C-18 Lichrocart cartridge, 4 mm diameter, 12 cm length, Merck Co.). The mobile phase consisted of 1% (v/v) acetic acid, 10% (v/v) methanol, and 89% (v/v) 5 mM sodium acetate. The flow rate was 1.2 ml/min. The effluent was monitored with a UV detector (model LC-3A, Shimadsu Co.) The analyte (tMA) and internal standard (vanillic acid) were eluted at 4.2-4.4 and 12.6-13.3 min, respectively. The detection limit was 0.05 mg/L. Urine tMA concentration was reported as mg/g creatinine.

Statistics. Results were expressed as means ± SD; the p value was calculated by unpaired t-test; p <0.05 considered statistically significant.

Results

As listed in Table 1, urine tMA levels in the group of gas station attendants averaged 4.00 ± 12.49 mg/g creatinine, which was significantly higher than the levels in the control group (0.116 ± 0.027 mg/g creatinine, p <0.05).

The group of gas station attendants included one subject with an exceptionally high urine tMA level (64.2 mg/g creatinine). No explanation of this high level was evident. However, even after exclusion of this outlier, the urine tMA levels in the gas station attendants (1.93 ± 5.66 mg/g creatinine) was significantly elevated in comparison to the controls.

Discussion

Because of its occurrence in mineral oil and its formation in many combustion processes, benzene

Table 1. Concentrations of trans,trans-muconic acid (tMA) in urine specimens from gas station attendants and non-exposed control subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>Urine tMA level* mg/g creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>49</td>
<td>0.116 ± 0.027 (0 - 0.40)</td>
</tr>
<tr>
<td>Gas station attendants</td>
<td>30</td>
<td>4.00 ± 12.49§ (0 - 64.2)</td>
</tr>
</tbody>
</table>

* mean ± SD, with range in parentheses
§ p <0.05 vs controls
is a widespread environmental pollutant. Since benzene is hemotoxic and has been classified as a human carcinogen, it is important to monitor and control exposure to benzene vapor in the environment and especially in workplace air [1,2].

In developing countries, there is growing recognition of the public health hazards of benzene exposure, but relatively few of these countries have policies, regulations, and programs to combat the problem. A main purpose of this study was to document the increased exposure to benzene in a population of low-skilled workers that is widely distributed throughout the developing world.

Gas station attendants can be classified as an occupation of low-skilled workers at high risk of benzene exposure. They are constantly in contact with petroleum products during their daily work. Compared to mechanics, skilled workers who were previously investigated [10], gas station attendants generally have a lower socioeconomic status. Like other migrants from the rural area to Bangkok [13], they have a relatively low educational background. Therefore, they need instruction about self-care and personal hygiene in relation to their work with hazardous chemicals. For these workers, protective equipment, such as gloves and masks, is necessary and should be provided. Specific policies and regulations regarding benzene exposures are needed for gas station attendants as well as for workers in other forgotten high-risk occupations.

References