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Research Article

Comparative Evaluation of Urinary Biomarkers of exposure to benzene

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ABSTRACT
Benzene, a known human carcinogen is an airborne pollutant of industrial and general environments. Although toxic effects of benzene at high exposures are well documented, the risk for adverse health effects at low levels of benzene exposure remains unknown; therefore identification of sensitive and specific biological markers is necessary for the definition of low benzene level exposure, so in this study a comparative evaluation of urinary biomarkers
(urinary trans, trans-muconic acid , S-phenyl mercapturic acid, and unmetabolized benzene) was carried out in order to characterize the best benzene exposure biomarker. With this aim, 40 policemen engaged in traffic control, 40 gas station workers and 40 occupationally non-exposed persons were investigated. Spot urine samples were obtained prior to and at the end of the work shift from each subject. Mean benzene exposure was 0.35, 0.22 and 0.09 ppm, respectively, with higher levels in gas stage workers than in control group. U-benzene showed a strong exposure-related increase. In conclusion, in the range of investigated benzene exposure, U-benzene is the marker of choice for the biological monitoring of occupational and environmental exposure.

Keyword: Benzene, Urinary biomarker, unmetabolized benzene

INTRODUCTION

Benzene, a known human carcinogen [1] (group 1 IARC) is a major chemical widely used all over the world in many industries. As a consequence of these production activities, it is found as an airborne pollutant in industrial environments [2]. Benzene is also distributed in the general environment by vehicles due to both fuel evaporation and gas-fueled engine emissions [3]. Despite the progressive reduction in the concentrations of benzene achieved in work and living environments in recent years, this aromatic hydrocarbon is still an occupational and airborne pollutant that arouses extreme toxicological concern, accurate estimation of human exposure to benzene is therefore needed. For the definition of exposure to low levels of benzene as well as for the evaluation of health risks posed by this exposure, the identification of suitable, specific, and sensitive biological markers is needed. Numerous studies have examined the suitability of potential biomarkers, on the basis of the evaluation of their relationship with external exposure [4-12]. T*rans*,*trans*- Muconic acid (*t*,*t*-MA, 2,4-hexadienedioic acid), *S*-phenyl mercapturic acid (SPMA, *N*-acetyl-*S*-phenyl-L-cysteine) and unmetabolized benzene in urine (U-benzene) have been proposed to assess lower levels of exposure to benzene. However, at concentrations below 0.1–0.5 ppm their validity has recently been questioned [7; 8; 13-15]. T,*t*-MA is very often used on a routine base, but at low exposure levels, its use is complicated by the fact that it is also a metabolite of the food preservative, sorbic acid (SA). The aim of this work was to compare the ability of t,t-MA,S-PMA, and U-benzene to detect occupational and environmental low benzene exposure. With this aim, gas station attendants, urban policemen, and controls working in Tehran, the capital of Iran, and the suburban areas surrounding it were investigated.

MATERIALS AND METHODS

Study population

Sixty healthy men from the city of Tehran, Iran, were enrolled in the study. The study population consisted of 120 traffic policemen engaged in traffic control and 40 gas station workers in five districts with medium to high traffic level, selected on the basis of traffic flow data recorded by the Regional Agency for Environmental Protection. For the measurement of any background levels originating from other activities such as car refueling, urine samples were collected from 40 occupationally non-exposed persons in the same districts acted as referents. All of the subjects were men between 27 and 57 (mean 41) years of age and none of them were smokers. Since there may be a significant risk of dermal exposure, it was requested from gas station workers to wear plastic gloves during the work shift.

Sampling

The exposure measurements were carried out in July 2013. Urine samples were collected at the beginning and at the end of the shift. Urine samples were stored in a cooling box until they were received by the laboratory,

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Table 1: Urinary concentration of benzene biomarkers before and after of work shift in (µg/L).

	Subjects (n)	Mean ± SD	Range (min-max)
Unmetabolized urinary benzene before work shift	Policemen (40)	0.09±0.061	0.0011-0.305
	Gas stage worker (40)	0.15±0.112	0.0028-0.883
	Controls (40)	0.007±0.001	0.0005-0.0076
Unmetabolized urinary benzene after work shift	Policemen (40)	0.201±0.173	0.0024-0.479
	Gas stage worker (40)	0.31±0.212	0.002-0.916
	Controls (40)	0.0009-0.005	0.0005-0.0321
Urinary S-PMA before work shift	Policemen (40)	0.48±0.114	0.131-0.706
	Gas stage worker (40)	0.60±0.477	0.09-2.63
	Controls (40)	0.21±0.178	0.1-0.792
Urinary S-PMA after work shift	Policemen (40)	0.54±0.211	0.140-1.64
	Gas stage worker (40)	0.73±0.518	0.230-3.09
	Controls (40)	0.31±0.173	0.09-0.710
Urinary tt-MA before work shift	Policemen (40)	46.11±10.84	19.38-82.51
	Gas stage worker (40)	54.03±18.05	30.25-98.74
	Controls (40)	33.86±12.10	20.14-63.28
Urinary tt-MA after work shift	Policemen (40)	55.62±17.02	27.14-90.07
	Gas stage worker (40)	64.75±19.40	36.58-110.39
	Controls (40)	47.01±13.67	23.56-75-41

where they were divided into several fractions and frozen at -20°C until analysis.

Analytical methods

urinary t,t-MA was determined by high-performance liquid chromatography and UV detection after pre-purification of urine with solid-phase extraction using a strong anion exchange column (300 mg, Supelco) according to a published procedure [16]. The detection limit of the procedure was 10 µg/L. Determination of urinary S-PMA was carried out by an immunoassay technique according to a previously published method [17]. The detection limit of the procedure was 0.2 µg/L. Determination of U-benzene was done by headspace solid-phase micro extraction followed by gas chromatography/ mass spectrometry analysis according to a published method [18] Briefly, 2 mL urine was poured in a 5-mL vial containing 150 mg NaCl. The internal standard solution of benzene-d6 (2 µL) in methanol (0.5µg/L) was added, and the vial was immediately sealing with a screw-top septum containing cap. Benzene was sampled from the urine headspace by the solid-phase micro extraction technique using a PDMS fiber. Analyte separation was done by gas chromatography which was fitted with a DB-1 capillary column (60 m, 0.25mm id, 0.25µm film thickness). Quantification was done using a mass spectrometry detector operating in the electron impact mode.

Airborne Benzene.

Airborne benzene was collected by Radiello passive samplers (Fondazione S. Maugeri, Padova, Italy) during part of the work shift (typically from 8:00 a.m. to 2:00 p.m). At the end of the monitored period, the adsorbed benzene vapors were desorbed by carbon disulfide and the solution was analysed using a gas chromatographic method with flame ionization detection.

Statistical Analyses

The results were statistically analyzed using SPSS for Windows version 18 (SPSS Inc, Chicago, IL). The relationship of benzene exposure and the urinary biomarkers was assessed by making comparisons among the occupational groups and between pre-shift and post-shift samples and by using correlation/regression techniques. A value corresponding to one-half of the detection limit was assigned to measurements below analytic detection. Because the distribution of data was not normal, the analysis was carried out by means of two statistical procedures: parametric methods on logarithm transformed variables (t test for independent or paired samples, ANOVA, and Pearson's correlation coefficient) and corresponding nonparametric techniques (Mann-Whitney, Wilcoxon, Kruskal-Wallis test, and Spearman's q).

RESULTS AND DISCUSSION

Urinary benzene (U-Benzene) was investigated as a biomarker of exposure among benzene-exposed workers and unexposed subjects in Tehran, Iran. Exposure to airborne benzene, as time-weighted average value, was assessed by using personal passive sampler. The results show that exposure to benzene in the three groups of workers (traffic policemen, gas station workers and occupationally non-exposed persons) was low (overall mean values being 0.35, 0.22 and 0.09 ppm, respectively) when compared to the ACGIH limit values (0.5ppm). In Table 1, data on personal exposure to airborne benzene, as time-weighted average value, excretion of t,t-MA, S-PMA, and U-benzene (before and after of work shift), in subjects divided according to job title are reported. Using parametric statistics, higher personal exposures were found in gas station workers, followed by traffic policemen, with comparable levels, and finally by the control group. This exposure rank is in agreement with what was expected on the basis of previous experience, although the concentrations measured in this study show a trend toward lower levels in comparison with the past. Statistically significant correlations were observed in all subjects between airborne benzene concentrations and levels of *t,t*-MA and S-PMA and benzene in urine (

Table 2. Pearson Correlation (*P* value) between urinary benzene biomarkers before work shift

	Urinary t,t-ma	Urinary S-PMA	Urinary Benzene	Airborne benzene
Airborne benzene	0.413(0.0001)	0.516 (0.0001)	0.629 (0.0001)	1
Urinary benzene	0.137 (0.125)	0.283 (0.011)	1	
Urinary S-PMA	0.200(0.125)	1		
Urinary t,t-ma	1			

Table 3. Pearson Correlation (*P* value) between urinary benzene biomarkers after work shift

	Urinary t,t-ma	Urinary S-PMA	Urinary Benzene	Airborne benzene
Airborne benzene	0.581(0.0001)	0.672 (0.0001)	0.759(0.0001)	1
Urinary benzene	0.403 (0.0001)	0.536 (0.0001)	1	
Urinary S-PMA	0.398(0.0001)	1		
Urinary t,t-ma	1			

Pearson's r = 0.58, 0.67 and 0.759,obtained on log-transformed data, respectively). As it was seen the best correlation was found between U-benzene and airborne benzene, with the highest r-values. Significant correlations of the biomarkers between each other, with the highest r-value (r up to 0.67), were found (Table 2 and 3). In consist with present study results; Suramya Waidyanatha et al. report that UB is a good biomarker for exposure to low levels of U-benzene.

CONCLUSION

In the last decade, several researches were carried out to evaluate personal exposure to benzene in different groups of workers who are occupationally or non-occupationally exposed to petrol engine exhaust fumes and/or to gasoline vapors [6; 19-30]. Several urinary metabolites (S-PMA, t,t-MA and phenolic metabolites) have been used to assess short-term exposures to benzene. However, their usefulness has been limited because of poor specificity (high background levels of the phenolic metabolites and t,t-MA) [31-34] lack of sensitivity or complexity of the assays (SPMA) [35]. By considering these problems, the determination of U-benzene presents a simple alternative biomarker for biological monitoring of benzene exposure. The relationship between U-benzene and airborne benzene from the current investigation is comparable with previously reported results.

In our study U-benzene was detected in all control subjects (Table 1), which was significantly lower than the mean among workers exposed occupationally to benzene. Among controls whom all of them were non-smokers the mean level of U-benzene was similar to that reported in the literature (Ghittoriet al. [36] and Kok and Ong [37]). Since there is no known endogenous source of benzene, this background of U-benzene among non-smokers points to ambient exposure to benzene. The detection of U-benzene in all (exposed and unexposed) subjects indicates that this assay can be used to monitor ambient as well as occupational exposure to benzene. Indeed, because U-benzene is derived exclusively from exposure to benzene, these results suggest that U-benzene is the optimal short-term biomarker of benzene.

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