NOTES

Effect of Simultaneous Exposure to Benzene and Ethanol on Urinary Thioether Excretion

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The toxicity of benzene is not an issue of the past, especially in developing countries. Bone marrow toxicity is demonstrated among workers. In this study, the effect of simultaneous exposure to benzene and ethanol on benzene metabolism in mice was investigated by measuring the excretion of thioethers in urine. Urinary thioether excretion significantly decreased in the mice receiving both benzene and ethanol compared with the animals receiving benzene only. The assay of determining thioethers in urine samples in this study is a simple and low-cost method, thus suitable for routine use, especially in developing countries, not only for benzene, but also for other alkilating agents, which can be found during occupational exposure. Our results suggest that further research is needed to elucidate the mechanisms of decreased urinary excretion of thioether after simultaneous exposure to benzene and ethanol.

benzene   ethanol   exposure   thioethers

1. INTRODUCTION

Aksoy, Erdem and Dincol reported an association between benzene and leukemia among shoeworkers in Turkey almost 40 years ago [1]. The International Agency for Research on Cancer classified benzene as an agent carcinogenic to human (group 1) [2]. Benzene has been widely used as a solvent, but its use is declining in most developed countries because it is a well-established carcinogen. Benzene is considered as a toxic impurity in other industrial solvents, like toluene and xylene. Therefore, benzene has been replaced with other less toxic organic solvents [3].

However, the toxicity of benzene is not an issue of the past, especially in developing countries [4]. Bone marrow toxicity has been demonstrated among workers, resulting from benzene exposure levels under 3.2 mg/m^3 (1 ppm), i.e., the current occupational limit in most developed counties [5]. Recently, the American Conference of Governmental Industrial Hygienists (ACGIH) reduced the benzene exposure limit to 1.6 mg/m^3 (0.5 ppm) [6].

The mechanisms of benzene toxicity continue to be actively researched today [7]. Benzene is metabolized mainly by ethanol inducible cytochrome P-4502E1 (CYP2E1) in animal and human liver microsomes [8, 9]. Toxic effects of benzene, acute as well as chronic, are the consequence of the unknown reactive metabolite [10].

Exposure to certain electrophilic agents, reacting with reduced glutathione (GSH), increases to-

tal thioethers detected after alkaline hydrolysis of urine. GSH conjugation results in the formation of cysteine conjugates, pre-mercapturic acids, mercapturic acids and other thioethers, which are excreted in urine [11]. Phenol is the main metabolite of benzene. The formation of phenol from benzene involves initially the formation of benzene oxide [12]. The conjugation of benzene oxide and glutathione forms a pre-mercapturic acid, which is further metabolized in kidneys yielding S-phenylmercapturic acid [13]. Recent studies confirm the usefulness of mercapturic acids as a biological exposure index for electrophilic chemicals [14]. Determining thioethers in urine samples is a noninvasive method for detecting occupational exposure to benzene and other alkilating agents.

In this study, the effect of simultaneous exposure to benzene and ethanol on benzene metabolism in mice was investigated by measuring the excretion of thioethers in urine.

2. MATERIAL AND METHODS

2.1. Animals

Albino BALB/C male mice, 2–4 months old (Pasteur Institute, Novi Sad, Serbia), which were kept in a natural dark–light cycle and fed with standard diet (Veterinarian Institute, Zemun, Serbia) and water ad libitum, were used.

2.2. Experimental Design

The experiment was carried out on four groups of mice, each comprising 5 animals. The controls received i.p. olive oil and saline. The animals in the experimental groups received i.p. ethanol (1.5 g/kg) and olive oil, or benzene (100 µl/kg) and saline, or both benzene and ethanol. After the treatment, urine samples were collected during 8 h to measure the total concentration of thioethers [15]. Metabolic cages for mice provided a good separation between urine and faeces.

Thioethers were determined with Ellman's method [16]. Free sulfhydryl (SH) groups react with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) to photometrically measurable thiol. Mercapturic acids and other thioethers were converted into the corresponding thiols with alkaline hydrolysis. Proteins were precipitated with perchloric acid. After centrifugation, sodium hydroxide was added to the supernatant. Alkaline hydrolysis was carried out in closed polypropylene tubes at 100 °C for 120 min. After cooling, the hydrolysate was neutralized in the cold (10 °C) to pH 7.2–7.8 with 5N hydrochloric acid. Ellman assay and DTNB were added to hydrolyzed urine samples and the increase in absorbance was measured at 405 nm on a Unicam Ultraviolet Spectrophotometer SP 1800 (Pye Unicam, UK). Total thioethers excreted in urine expressed in millimoles (calculated by multiplying the concentration of thioethers in urine by the volume of urine) were divided by animal body weight expressed in grams for each animal.

After the urine samples were collected, all the animals were sacrificed. The liver was removed, blotted on filter paper, weighed and then homogenized in an electric homogenizer in a glass vessel with a Teflon pestle. The liver homogenate was used to measure reduced glutathione content [17]. Glutathione content was normalized to liver weight and animal body weight.

The Animal Ethics Committee of the Faculty of Medicine, Novi Sad, Serbia, approved the experimental protocol

2.3. Statistical Analysis

The statistical significance of the results was analyzed with Student's t test; $p < .05$ was considered significant.

3. RESULTS

Table 1 shows our results of glutathione content in the liver related to liver weight (µmol/g·L) and to body weight (nmol/g b.w.) in the control group, and in mice treated with ethanol, benzene, and benzene and ethanol. These results do not show any statistically significant differences in glutathione content in the liver normalized either to liver weight or animal body weight, between the tested groups of animals.

TABLE 1. Content of Glutathione in the Liver Related to Liver Weight (µmol/g·L) and to Body Weight (nmol/g b.w.) in the Control Group, and in Mice Treated With Ethanol, Benzene, and Benzene and Ethanol

Group of Mice	Glutathione in Liver ($M \pm SD$)	
	(µmol/g·L)	(nmol/g b.w.)
Control	6.52 ± 1.14	427.67 ± 168.54
Ethanol	5.43 ± 0.47	275.71 ± 26.92
Benzene	5.95 ± 0.72	290.54 ± 29.43
Benzene + ethanol	5.95 ± 1.25	293.54 ± 59.51

Table 2 shows the concentration of thioethers in the animals' urine. The concentration of thioethers in urine normalized to animal body weight in the group of mice treated with both benzene and ethanol was significantly lower than in mice treated with benzene only.

TABLE 2. Concentration of Thioethers in Urine, Normalized to Body Weight (µmol/g b.w.) in the Control Group, and in Mice Treated With Ethanol, Benzene, and Benzene and Ethanol

Group of Mice	Thioethers in Urine (µmol/g b.w.) ($M \pm SD$)
Control	3.61 ± 1.37
Ethanol	3.85 ± 1.84
Benzene	5.33 ± 1.81
Benzene + ethanol	3.21 ± 0.74*

Notes. *$p < .05$ compared to benzene; $n = 5$ per group.

4. DISCUSSION

Urinary excretion of thioethers significantly decreased in the mice receiving both benzene and ethanol compared with the animals receiving benzene only. The applied dose of ethanol decreased urinary excretion of thioethers, but glutathione content in the liver did not change.

The assay for determining thioethers in urine samples in this study is a simple and low-cost method. Thus, it is suitable for routine use, especially in developing countries, not only for benzene, but also for other alkilating agents, which can be found during occupational exposure. Mercapturic acids refer to a toxicologically relevant absorbed dose, while other urinary metabolites usually refer to the absorbed dose only [18].

Diet [19, 20] and tobacco smoke [21, 22] can affect urinary excretion of thioethers. There is considerable interindividual variation when diet, a major source of sulfur-containing compounds, is not controlled [19, 20]. Cigarette smokers excrete significantly higher levels of thioethers than nonsmokers [21, 22]. Smoking is a significant source of exposure to benzene in both occupational and nonoccupational groups [23]. The smoking habit strongly influences the metabolism of benzene [24, 25]. However, in this experiment, diet factors were kept under strict control and there were no other influences such as tobacco smoke or other xenobiotics. The only treatment was with benzene and/or ethanol. On this basis, we concluded that the observed difference in thioethers excretion was the consequence of the treatment.

Urinary phenol excretion was found to be inhibited by a dose of 1.5 g/kg of ethanol in benzene-treated mice (100 µl/kg) [26]. Our hypothesis is that both phenol and thioethers decreased in urine due to competition for CYP2E1, which is required for the metabolism of both benzene and ethanol. Acute ethanol ingestion inhibits benzene oxidation in the liver, reacting with an ethanol-inducible form of cytochrome P-450 [27]. In contrast with the stimulating effects of chronic consumption, acute ethanol intake inhibits the metabolism of other xenobiotics [28].

The importance of the isoform CYP2E1 of the human cytochrome P-450 superfamily of enzymes for occupational medicine is derived from its broad spectrum of the substrates that include organic solvents and industrial monomers. A long-term consumption of ethanol induces the metabolism of many organic solvents [9, 29]. In view of the opposite effects of the immediate and long-term consumption of alcohol beverages, the history of acute and chronic intake of ethanol should be carefully evaluated in workers occupationally exposed to benzene. The possible consequences are related to individual presentation of clinical symptoms of toxicity and to the interpretation of the results of biological monitoring.

Our results suggest that further research is needed to elucidate the mechanisms of decreased urinary excretion of thioethers after simultaneous exposure to benzene and ethanol.

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