Short Communication

Effect of Simultaneous Exposure to Benzene and Ethanol on Urinary Phenol Excretion in Mice

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Benzene has been widely used as a solvent, but its use is declining in most developed countries because it is a well established human carcinogen. An association between occupational exposure to benzene and health effects such as aplastic anaemia and leukaemia has been reported in studies of workers in various industries¹. For many solvent uses benzene has been replaced by other less toxic organic solvents and is considered a toxic impurity in other industrial solvents², but it is still used as a starting material in numerous chemical syntheses. In addition to industrial sources, benzene is present in the environment as a component of cigarette smoke and automobile emissions³. Benzene induces hematotoxicity as a result of chronic exposure. Acute exposure to benzene causes neurotoxic and hepatotoxic effects. Toxic effects of benzene, chronic as well as acute, are the consequence of the unknown reactive metabolite produced by cytochrome P-450⁴. Benzene is metabolized mainly by ethanol inducible cytochrome P-4502E1 (CYP2E1) in animal and human liver microsomes^{5–7}.

Ethanol is consumed worldwide in tremendous amounts and is an effective inducer of hepatic xenobiotic metabolism, especially involving pathways accomplished by the isoform CYP2E1 of cytochrome P-450. Therefore, whenever xenobiotics that are substrates of CYP2E1, such as many organic solvents, are taken in by an individual who is also chronically consuming ethanol, the accelerated metabolism of these agents has to be considered⁸. In contrast to the long-term consumption of ethanol, which induces the hepatic metabolism of xenobiotics, short-term consumption inhibits their metabolism because of direct competition for CYP2E1⁹.

Ethanol, when given to a living body, exerts dual effects on the xenobiotic metabolizing enzymes, i.e., inhibition and stimulation. Which one is more predominant over the other depends on the time that has elapsed after ethanol ingestion. In an early period when ethanol exists in the body in high concentrations, it may preferentially act as an inhibitor^{10, 11}.

In this study, effect of simultaneous exposure to benzene and ethanol on benzene metabolism in mice were investigated by measuring the concentration of phenol, the main metabolite of benzene.

Materials and Methods

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

Experimental design. The experiment was carried out on four groups of mice, each comprising 5 animals. The control group 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 that urine samples were collected for 8 h to determine the phenol concentration by gas chromatography (the detection limit for this method was 10 µmol/l)¹². To obtain good separation between urine and faeces, mice metabolic cages were used. Concentrations of phenol in urine were related to animal body weight. Total phenol excreted in urine expressed in nmol (calculated by multiplying the concentration of phenol in urine by the volume of urine) was divided by the animal body weight expressed in grams for each animal.

After collecting urine samples, 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 total cytochrome P-450¹³ and protein content¹⁴.

Statistical analysis. The statistical significance of the results was analyzed by Student t-test, and $p < 0.05$ was considered significant.

Results

Cytochrome P-450 and protein content in liver showed no statistically significant differences.

The results obtained for the measured concentrations of phenol in the urine of the animals are shown in Table 1.

The concentration of phenol in urine related to animal body weight in the group of mice treated with both benzene and ethanol was significantly lower than in mice treated only with benzene.

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Table 1. Concentration of phenol in urine related to body weight (nmol/g b.w.) in control group, and mice treated with ethanol, benzene and benzene + ethanol

Group of mice	Phenol in urine (nmol/g b.w.)
Control	not detected
Ethanol	not detected
Benzene	178.09 ± 32.00
Benzene + ethanol	14.28 ± 2.83**

** - $p < 0.01$ compared to Benzene. not detected - the phenol concentration in urine was less than the detection limit.

Discussion

Urinary phenol excretion was significantly decreased in the mice receiving both benzene and ethanol compared with the animals receiving only benzene. This dose of ethanol decreased the urinary excretion of phenol, but did not change either total cytochrome P-450 or protein content in liver. Phenol is the main metabolite of benzene^{15, 16}. Acute ethanol ingestion inhibits benzene oxidation in the liver by reacting with the ethanol inducible form of cytochrome P-450^{5, 10}. The metabolism of benzene was found to be inhibited by this dose of ethanol. This is probably due to competition for cytochrome P-450E1, which is required for the metabolism of both benzene and ethanol^{5, 9}.

It is well recognized in industrial and environmental health that man is exposed simultaneously to more than one chemical. Moreover, the metabolism of chemicals may be modified by social habits¹⁷. Workers occupationally exposed to organic solvents take alcoholic beverages occasionally or habitually. In view of the opposite effects of immediate and long-term alcohol consumption, the net effect of concomitant alcohol use and xenobiotic exposure in a long-term alcohol consumer is difficult to predict⁹. Ingestion of ethanol is therefore a noticeable source of error in the biological monitoring of benzene uptake. That is why the history of ethanol intake should be carefully evaluated in workers occupationally exposed to benzene and other organic solvents.

On the other hand, the study presented here is only preliminary and long-term research is needed to elucidate the mechanisms of decreased urinary phenol excretion after simultaneous exposure to benzene and ethanol.

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