EFFECT OF BENZENE ON DRUG METABOLIZING ENZYME ACTIVITIES OF LUNG AND LIVER MICROSOMES

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SUMMARY: In vivo treatment of rabbits with benzene (880 mg/kg, s. c., 3 days) caused 7. I-fold increase in hydroxylation of p-nitrophenol to 4-nitrocatechol in liver microsomes. A 3.7 fold increase was also observed in liver aniline 4- hydroxylation activities. Benzene treatment did not cause any significant changes on drug metabolizing enzyme activities of lung microsomes. Although benzene treatment did not change cytochrome P-450 contents of lung and liver microsomes, it caused 1 nm spectral shift in the absorption maxima of CO difference spectrum of the dithionote reduced liver cytochrome P-450 forming cytochrome P-451.

Index Words: Benzene, drug metabolizing enzymes, mixed-function oxideses, microsomes, benzphetamine N-demethylase, aniline 4-hydroxylase, P-nitrophenol hydroxylase, cytochrome P-450.

INTRODUCTION

Many industries use benzene extensively as a solvent or as a starting material for chemical syntheses. In recent years, the consumption of benzene has increased and it has become the major industrial pollutant due to the replacement of tetraethyl lead in gasoline by benzene which also has anti-knock properties.

The major toxic effect of benzene is hemapoietic toxicity. Chronic exposure of humans of low levels and of animals to high concentrations result in lymphocytopenia, thrombocytopenia, and pancytopenia or aplastic anemia (1,12). Benzene is also a carcinogen. Several researchers have noted that it is associated with the increased incidence of acute myelogenous leukemia in humans (1,2,12).

Recent studies with benzene strongly indicate that metabolism of benzene is required for its hemapoietic toxicity (12), and that benzene is metabolized by cytochrome P-450 dependent drug metabolizing enzymes, also called mixed-function oxidases, of liver and bone marrow (12).

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A wide variety of structurally unrelated drugs and xenobiotics and endogenous compounds such as fatty acids, sex steroids, cholesterol, bile acids, and prostaglandins are metabolized by drug metabolizing enzymes of the endoplasmic reticulum in a variety of tissues (5,14,21). This system serves as a route of detoxification and, in contrast, also as a route of metabolic activation to yield reactive metabolites which initiate toxic and carcinogenic events (12,14).

It has been shown that in vivo treatment of animals with benzene induces its own metabolism in liver and bone marrow (12). However, liver plays an essential role in bioactivation of benzene rather than bone marrow (12). Since a variety of drugs and xenobiotics have been metabolized by the same drug metabolizing enzyme system, from biochemical and pharmacological viewpoint, it is important to know the effects of benzene treatment on drug metabolizing enzyme activities. This study is undertaken to investigate the effects of benzene treatment on microsomal aniline 4-hydroxylase, benzphetamine N-demethylase and P-nitrophenol hydroxylase activities of liver and lung which have not been addressed before.

MATERIALS AND METHODS

Isolation of Microsomes

The animals used in the experiments were male New Zealand white rabbits (2.4-3.3 kg). Benzene (880 mg/kg body weight) was injected subcutaneously once daily for 3 days and the animals were killed 24 hours after the last treatment. Liver and lung microsomes were prepared from the individual rubbits immediately after killing by differential centrifugation as described in detail before (4,5,17). All the subsequent steps were carried out at 0-4°C. The microsomes were washed by homogenization, in 0.15 M KCI containing 1 mM EDTA, collected by centrifugation, suspended in 25% glycerol containing 1mM EDTA. The enzymatic reaction rates and cytochrome P-450 concentration in microsomes were determined with these freshly made preparations. Additional microsomal suspensions in small aliquats were gassed with nitrogen and were immediately immersed into a tank containing liquid nitrogen and were kept in liquid nitrogen upto two weeks.

Analytical Procedures

The protein content of microsomes were determined by the procedure of Lowry *et al.* (13) using bovine serum albumin as a standard.

Benzphetamine N-demethylase activity was determined by measuring the quantity of formaldehyde formed according to the method of Nash (16) as modified by Cochin and Axelrod (8). NADPH generating system was used as a cofactor. Typical assay mixture contained 1.5 mM benzphetamine HCI, 100 mM potassium phosphate buffer pH 7.7, NADPH generating system consisting of 0.5 mM $NADP^+$, 2.5 mM $MgCl_2$, 2.5 mM glucose G-phosphate dehydrogenase, 14.6 mM HEPES buffer pH 7.8 and 1 mg of liver and lung microsomal protein in a final volume of 1.0 ml.

Anilline 4 hydroxylase activity was measured by the quantitation of P-aminophenol. Details of assay conditions were given by Arinç and Iscan (4). NADPH generating system described above was used as a cofactor. One mg of liver microsomal protein or 2 mg of lung microsomal protein was used each assay.

The hydroxylation of P-nitrophenol to 4-nitrocatechol (1,2-dihydroxy-4-nitrobenzene) was determined as described by Reinke *et al.* (19). The assay conditions were optimized in our laboratory. The reaction mixture, in a final volume of 1.0 ml, contained 100 mM Tris-HCI buffer pH 6.8, p-nitrophenol, 0.125 mM for liver, 0.250 mM for lung, mirosomes (1.5 mg for lung and control liver, and 0.5 mg for treated liver) and 0.5 mM NADPH generating system described above.

The procedures used for cytochrome P-450 and cytochrome P-450 reductase assays have been described elsewhere in detail (5, 11).

Liver P-450 was solubilized by treatment of microsomes with sodium cholate (3,5). The solubilized microsomal fraction was fractionated with ammonium sulfate. These fractions precipitating between 35 and 50 % saturation of the supernatant solution with ammonium sulfate contained cytochrome P-450 and was separated from cytochrome b_5.

Chemicals

NADPH, bovine serum albumin, horse hearth cytochrome c were purchased from Sigma Chemical Co., St Louis, Mo., USA, Glycerol and p-nitrophenol were the products of E. Merck. Benzphetamine HCI was a gift from Dr. James F. Stiver of Up John Co., Kalamazoo, MI., USA. All other chemicals were of analytical grade and were obtained from commerical sources at the highest grade of purity available.

RESULTS AND DISCUSSION

Since the optimum conditions for liver and lung aniline 4-hydroxylase and benzphetamine N-demethylase were established in previous studies (4,6), our efforts in this study are concentrated on the determination of the effects of pH, microsomal protein amount, and substrate concentrations for lung and liver microsomal p-nitrophenol hydroxylase activities. The maximal activities were noted at pH 6.8 for both tissues. The rates of p-nitrophenol

Table 1: Effects of Benzene on Liver and Lung Microsonal Enzyme Activities and Cytochrome P-450 Levels of Rabbits.

Enzymes of P-450	LIVER[a]		LUNG[a]	
	Control	Benzene	Control	Benzene
p-Nitrophenol hydroxylase[b]	0.20-0.04 (3)	1.42-0.21* (4)	0.26-0.06 (3)	0.18-0.03 (5)
Aniline 4-hydroxylase[b]	0.43-0.04 (3)	1.61-0.04* (3)	0.28-0.07 (3)	0.24-0.04 (5)
Benzphetanine N-demethylase[b]	7.75-0.47 (3)	6.53-0.67 (4)	12.9-2.34 (3)	11.53-2.37 (5)
P-450 Reductasbe[b]	77-13 (3)	65-3 (4)	81-30 (3)	86-14 (5)
Cytochrome P-450[c]	0.76-0.15 (4)	0.95-0.15 (3)	0.29-0.15 (4)	0.17-0.03 (4)

a Values in paranthesis represent the number of tissues.

b Values are nanomoles of the respective product formed/min/mg microsonal protein+SD

c Values are nanomoles cytochrome P-459/mg microsonal protein+SD

* Significantly different from control, p 0.01

hydroxylation activities were linear upto 2 mg/ml of incubation mixture for both control liver and lung and treated lung microsomes while the enzymatic rate of treated liver microsomes were linear upto 0.6 mg protein/ml of incubation mixture.

The effects of p-nitrophenol concentration on lung and liver microsomal hydroxylase activities were determined. The maximal rates of hydroxylation were obtained at a substrate concentration of 0.125 mM with liver microsomes obtained from either control or treated animals. With higher concentration of p-nitrophenol, substrate inhibition was observed. When lung microsomes of benzene-treated or control rabbits were used, maximal rates of 4-nitrocatechol formation was observed with 0.25 mM p-nitrophenol.

The induction of benzene hydroxylase, a member of drug metabolizing enzymes, were first reported by Snyder and coworkers (20). However, benzene cannot be considered as a general inducer of drug metabolizm since some activities such as ethylmorphine 0-dealkylase (9) and aminopyrine N-dimethylase (18) activities have been reported unchanged.

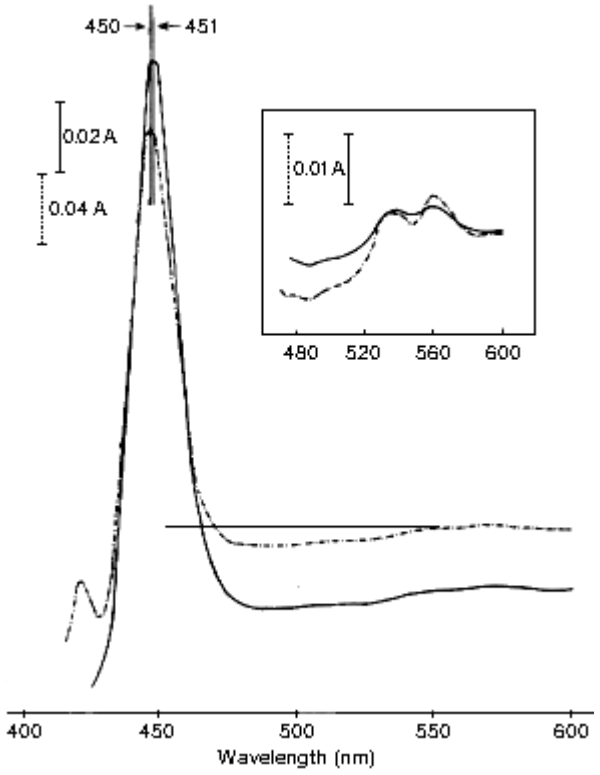

Figure 1: CO-difference spectra of dithionite reduced cytochrome P-450 of liver microsomes from control (- , -,) and benezene treated (- - - -)rabbits. Cytochrome P-450 and cytochrome P-451 contained 2.9 nmol and 1.9 nmol cytochrome per ml, respectively.

As seen in Table 1, benzene treatment caused about 3.7-fold increase in p-hydroxylation of aniline in rabbit liver microsomes. Benzene treatment also significantly increased the rates of liver p-nitrophenol hydroxylation. No effect was detected with benzene treatment on liver bezphetamine N-demethylase and cytochrome P-450 reductase activities. Pretreatment of rabbits with benzene did not cause any significant changes on the drug metabolizing enzyme activities of lung microsomes.

Although pretreatment with benzene did not effect total cytochrome P-450 content of lung and liver microsomes, the spectral properties of the solubilized liver cytochrome P-450 from benzene treated rabbits were found to be different than those of control rabbits. As seen in Figure 1, benzene pretreatment resulted in a shift of maximum absorption of the solubilized microsomal cytochrome P-450 from 450 nm to 451 nm in the CO-difference spectrum of dithionite reduced microsomes.

Cytochrome P-450 purified from liver microsomes treated with benzene has been shown to have identical N-terminal amino acid sequence as that of cytochrome P-450 LM3a induced by ethanol (10). It has also been shown that dithionite-reduced cytochrome P-450-CO complex of P-450 LM3a purified from ethanol treated rabbit livers showed a maximum absorbance at 452 nm. This type of P-450, the ethanol inducible isozyme, is especially active in the oxidative metabolism of alcohols, aniline and p-nitrophenol (15).

Although the spectrum of solubilized cytochrome P-450 given in Figure 1 can be a mixture of at least two cytochromes, the formation of 451 nm peak clearly indicates the synthesis of a new type of P-450.

Significant induction of both aniline and p-nitrophenol hydroxylase activities as well as the spectral properties of cytochrome P-450 suggest that the benzene inducible isozyme, like the ethanol inducible isozyme, may have a role in the formation of potentially toxic catechols.

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