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Effects of Toluene on Cytochrome P-450 Mixed Function Oxygenase and Glutathione-S-transferase Activities in Rat Brain and Liver

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Toluene is used as a solvent in industries for paint, lacquer, enamel, adhesives and varnish and as an intermediate for the synthesis of various other chemicals. Toluene vapours have been reported to produce a variety of changes in human individuals ranging from irritability and diminished psychomotor performance at low level of exposure, to disorientation and unconsciousness at high level exposure by LURIE (1949), LONGLEY et al. (1967) and LINDSTROM (1973). The chronic occupational exposure of toluene may in the final event cause organic dementia. Toluene toxicity has been reviewed in NIOSH publication (1973) and recently by HAYDON et al. (1977).

The toxicity of any compound besides time and level of exposure also depends upon its metabolism in the body. The so-called detoxifying enzymes like cytochrome P-450 (E.C. 1.14.14.1) and glutathione-S-transferases (E.C. 2.5.1.18) metabolize the toxic compounds to more hydrophilic substances. Since toluene seems to be more neurotoxic than hepatotoxic, it was ad hoc tempting that proportionally more metabolic transformation activities are occurring in brain than in liver tissue of animals exposed to toluene. In order to evaluate the theory, mixed function oxygenase and glutathione-S-transferase enzymatic activities were determined in rats after intraperitoneal injections of toluene.

MATERIALS AND METHODS

Chemicals. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase (270 units/mg protein), p-chloro-N-methylaniline, reduced glutathione and 1-chloro-2,4-dinitrobenzene were purchased from Sigma Chemical Company, St. Louis, MO, USA. NADP was obtained from Boehringer, Germany and fluorescamine from La Rosche Inc., New Jersey. All other chemicals were of reagent grade.

Animals and tissue processing. Young female Wistar rats SPF weighing between 74-80 g were used. The animals were injected intraperitoneally daily for 21 days with toluene (1 mL/kg) in corn oil (1:1, v/v). The control animals received the same amount of corn oil. The animals were reared in silent room with 80% humidity, 23°C temperature and 12 h of light. They were killed by decapitation 24 h after the last dose. The animals were fasted for 20 h before being killed but given water ad libitum. The animals were decapitated under light ether anesthesia and their livers and brains were immediately taken out and kept in ice-cold 0.25 M sucrose solution. All the following steps were also carried out at 0-4°C. The

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tissues were homogenized in 10 volumes of 0.25 M sucrose solution and the homogenate was fractionated by the method of HOGEBOOM (1955). Microsomes and cytosol were separated by first centrifuging the homogenate at 10,000 g-av for 20 min and recentrifuging the supernatant at 105,000 g-av for 60 min in an I.E.C./B-60 ultracentrifuge. The microsomal pellet was washed by resuspending in 0.25 M sucrose solution and recentrifuging it at 105,000 g-av and the pellet was finally suspended in 0.25 M sucrose/0.05 M tris-Cl buffer, pH 7.5.

ASSAYS

Mixed function oxygenase. This enzymic activity was assayed as N-demethylation of p-chloro-N-methylaniline by the method of VAN DER HOEVEN (1977). p-Chloroaniline was used as standard and the fluorescence was measured in a spectrofluorimeter with excitation wave length of 410 and emission wave length of 500 nm. The activity is indicated in specific units and one unit is equal to 1 nmole of p-chloroaniline formed/min/mg protein at 37°C under the conditions of assay.

GSH S-transferase. This activity was measured as the transformation of 1-chloro-2,4-dinitrobenzene in 105,000 g supernatants, i.e. cytosol fraction prepared from rat liver and brain. The method of HABIG et al. (1974) was employed. Specific activity is expressed in nmole conjugate formed/min/mg protein.

The protein concentration was measured in microsomes and cytosol by following the method of LOWRY et al. (1951).

RESULTS AND DISCUSSION

Table 1 shows that toluene intoxicated rats do not gain body weights to the same extent as the control. The per cent increase in control animals is 91.4 and in intoxicated rats 56.5. Therefore toluene intoxicated rats show 35% decrease in their body weights.

Cytochrome P-450 dependent N-demethylation of p-chloro-N-methylaniline increases in liver after intoxication with toluene. The results are represented in Table 2. The per cent increase is 33.8. In liver glutathione-S-transferase activity also increases but only 17.1%. In our experiments brain does not show any significant change in cytochrome P-450 activity but GSH S-transferase activity increases by 10.3%. MUNGIKAR & PAWAR (1976) have shown decrease in cytochrome P-450 after oral administration of toluene (0.72 mL/kg) for 2 days but ELOVAARA et al. (1979) have found increase in the same activity in rat liver. Our results of cytochrome P-450 are in agreement with the results of ELOVAARA et al. (1979). These authors did not study the GSH S-transferase activity in toluene intoxication.

It is well known that polycyclic carcinogens are initially converted into epoxides by microsomal cytochrome P-450 MFO and

Table 1. Effects of toluene on body weights.

Animals	Number of animals	Body weights Initial	Final	% Increase in body weight
Control	5	76.75±2.5	146±16.8	91.4
Experimental	5	78.25±2.36	122.5±9.88	56.5

Table 2. Effects of toluene on cytochrome P-450 and glutathione S-transferase activities.

Animals	Cytochrome P-450	% Increase in P-450	GSH S-transferase	% Increase in GST
Control	Liver 0.59±0.03		1138±66	
	Brain 0.028±0.001		154±12	
Experimental	Liver 0.79±0.01	33.8	1333±32	17.1
	Brain 0.029±0.001	3.5	170±3	10.3

Values are given as means of product formation in nmoles/min/mg protein ± S.D.

For each experiment five animals were individually assayed.

then epoxide may be transformed by three different routes (i) by conjugation with glutathione catalysed by GSH S-transferase, (ii) by hydrolysis catalysed by hydrase which convert the compounds into dihydrodiols, and (iii) by monoenzymatic hydrolysis transforming into phenols. For the metabolism of toluene, BAKKE & SCHELINE (1970) have shown that it is rapidly metabolized through oxidation to benzoic acid and to a lesser extent to p-cresol. GARNER (1976) has indicated that latter metabolic pathway requires arene oxidation with a possible formation of an epoxide intermediate. In our experiments we traced induction of cytochrome P-450 as well as GSH S-transferase in liver, therefore we assume that some part of toluene in liver is first converted into an epoxide by cytochrome P-450 and then some part of epoxide is detoxified through GSH conjugation, catalysed by GSH S-transferases. Our findings in brain show that during toluene intoxication, the mixed function oxygenase activity does not change but the glutathione-S-transferase activity increases by 10.3%. It caused the increase in ratio of the transferase activity to the mixed function oxygenase activity from 5500 to 5862 opposite to liver tissue where the ratio decreased from 1928 to 1687. These changes may ad hoc argue that the reactive electrophilic intermediates formed by the mixed function oxygenase are metabolized faster in brain than in liver tissue. Thus the changes in metabolizing

activities do not argue that brain tissue is more prone to toluene intoxication than liver tissue. On the other hand the composite cellular structure of brain tissue may cause that the two enzymic activities are topographically localized in different cellular compartments. SAVOLAINEN (1978) in his studies on distribution and nervous system binding of intraperitoneally injected toluene has shown that in brain the content of toluene decreases very rapidly as compared to liver so either it may not induce the enzyme to such extent as in liver or the metabolism in brain may be different than liver.

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REFERENCES

BAKKE, O. M. & R. R. SCHELINE: Toxicol. Appl. Pharmacol. 16, 691 (1970).
ELOVAARA, E., H. SAVOLAINEN, P. PFAFFLI & H. VAINIO: Arch. Toxicol., Suppl. 2, 345 (1979).
GARNER, R. C.: Progress in drug metabolism. Vol. 1, John Wiley & Sons, London (1976).
HABIG, W. H., M. J. PABST & W. B. JAKOBY: J. Biol. Chem. 249, 7130 (1974).
HAYDEN, J. W., R. G. PETERSON & J. V. BRUCKNER: Clin. Toxicol. 11, 549 (1977).
HOGEBOOM, G. M.: Methods in enzymology, Part I. Academic Press, New York (1955).
LINDSTROM, K.: Work environ. Health 10, 151 (1973).
LONGLEY, E. O., A. T. JONES, R. WELCH & O. LOMAEV: Arch. Environ. Health 14, 481 (1967).
LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR & R. J. RANDALL: J. Biol. Chem. 193, 265 (1951).
LURIE, J. B.: S. Afr. med. J. 23, 233 (1949).
MUNGIKAR, A. M. & S. S. PAWAR: Bull. Environ. Contam. Toxicol. 15, 198 (1976).
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (1973).
SAVOLAINEN, H.: Acta Pharmacol. Toxicol. 43, 78 (1978).
VAN DER HOEVEN, Th.: Anal. Biochem. 77, 523 (1977).