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BLOOD CHOLINESTERASE ACTIVITIES AFTER MALATHION POISONING IN LIVER INJURY

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Abstract : The present study was conducted on rats exposed to LD_{50} and sublethal doses of malathion for acute and chronic toxicity in the presence and absence of carbon tetrachloride ($CC1_4$) induced liver injury. In acute malathion poisoning either in the presence or absence of $CC1_4$, erythrocyte cholinesterase (EChE) and plasma cholinesterase (PChE) declined significantly. Correlation between toxic signs and enzyme activities existed in the early phase of exposure (10 min). Acute treatment of $CC1_4$ and malathion did not significantly alter aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. In chronic poisoning, the blood cholinesterases (ChE) were significantly lowered with corresponding elevation of AST and ALT which was noticed for 2 wk after the withdrawal of malathion. The findings suggest that the persons with pre-existing liver diseases may exhibit enhanced toxicological responses to pesticides.

Key words : cholinesterase malathion $CC1_4$ hepatotoxicity

INTRODUCTION

Malathion, an organophosphorus compound (OPC) inhibits erythrocyte cholinesterase (EChE) and plasma cholinesterase (PChE). Low activity of cholinesterases are observed in conditions like liver disorders, cancer, genetic variation, malnutrition, and also in pesticide poisoning (1, 2). Hence, estimation of these enzymes is useful in pesticide poisoning, cirrhotic patients and in liver transplant cases (3, 4). Liver injury, caused by toxic chemicals, illicit liquors and certain drugs, have been recognised as a toxicological problem (5). Farmers and industrial workers with liver disorders may be exposed to OPC poison during handling, manufacturing, packing or spraying.

The experimental study was designed to determine the altered toxicological

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responses of malathion in rats with acute and chronic liver injury induced by carbon tetrachloride (CCl_4). Further, it was aimed to correlate the ChE activities and liver functions with the toxic symptoms and the recovery pattern.

METHODS

Albino male rats weighing 120–150 g were procured from Indian Institute of Science, Bangalore. They were divided into 8 groups and housed 4 per cage with free access to food and tap water. The animals were allowed to acclimatize to room temperature for at least 1 wk prior to use.

Treatment of animals:

LD_{50} of malathion (Hindustan Pesticides, Kerala) in normal (1.74 g/kg) and CCl_4 (1.32 g/kg) treated rats were established. Fresh dilution of malathion was made on the day of the experiment. The animals were deprived of food for 2 hr prior to the oral administration of malathion and feeding was resumed 4 hr after the treatment. Rats were treated as follows (6).

Acute treatment:

G1. Control rats (distilled water or liquid paraffin, ip)

G2. Single oral LD_{50} of malathion (1.74 g/kg).

G3. Single i.p dose of CCl_4(1 ml/kg).

G4. After 24 hr of CCl_4 treatment, LD_{50} of malathion administered, (1.32 g/kg).

Blood samples were collected from the orbital sinuses in EDTA tubes at the intervals of 0, 2, 4, 24, 48, 72, 96, and 120 hr for the analysis of ChE activities at 405 nm (6). At end of the experimental period, blood was drawn from the heart for analysis of asparate aminotransferase (AST) and alanine aminotransferase (ALT). After sacrificing, the liver was dissected out and preserved in 10% formalin for histopathological studies.

Chronic treatment

G5. Control rats (distilled water or liquid paraffin, ip)

G6. Three different sublethal doses of oral malathion (100, 200 and 400 mg/kg) once a day for 14 days.

G7. CCl_4 i.p. once a day (0.1 ml/kg) for 14 days.

G8. CCl_4 i.p. once a day (0.1 ml/kg) for 14 days. After 48 hr of CCl_4 treatment, three different doses of malathion (100. 200 amd 400 mg/kg) were administered as in G6.

At end of the experimental period (on the 14th day and 28th day), rats were lightly anaesthetised with ether and blood samples were collected from the heart for analysing the enzyme activities.

Toxicity studies:

After oral administration of malathion, all the rats in acute studies were observed for toxic signs and were graded for severity of toxicity (7).

Mild (muscarinic) : diarrhoea, urination, miosis, bronchospasm, excess salivation and lacrimation.

Moderate (nicotinic) : fasciculations, tremor and jerks.

Severe (CNS) : convulsions, respiratory paralysis and death.

$CC1_4$ was diluted with equal volume of liquid paraffin (1:1) and was given i.p. at the dose of 0.2 ml/kg to $CC1_4$ control group. while normal control rats received 0.2 ml/kg of liquid paraffin (8–10).

Enzyme assay :

Cholinesterase : A portion of whole blood (0.2 ml) was diluted with buffer (1.8 ml). A 0. 02 ml of diluted blood was used for estimation of total ChE activity. Rest of the diluted blood was centrifuged at 4000 rpm for 15 min to separate out the plasma samples for the assay of PChE activity. Diagnostic kits of Boehringer Mannheim, Germany were used and the reading was made on autoanalyser (B.M. Hitachi, Model # 704) at 25°C. Buffer from the kit (3 ml) was mixed with 0.02 ml of whole blood/ plasma in the 5 ml cuvette. Substrate (0.1 ml) was added to the cuvette and the activity was measured for 5 min. EChE activity was obtained by deducting the PChE activity from the total ChE activity.

AST and ALT :

AST and ALT were analysed by using diagnostic kits (Fisher Inorganic and Aromatic Ltd., Madras) as described by Reitman and Frankel (11)

Statistical analysis:

The results were analysed by ANOVA. Paired 't' test was used to analyhse the data in acute experiments, as the same rat served as control.

RESULTS

Acute studies :

Rats, in groups 2 and 4 developed toxic symptoms within 5 min of administration of malathion. No abnormality was detected in the histological studies of livers. Rest of the rats in 2 and 4 groups recovered within 2½ hr. There was a significant decrease in ChE activities ($P<0.001$) with moderate toxic symptoms at 2 hr. Blood samples were not collected between 2–4 hr, as both the orbital sinuses had been punctured at 0 and 2 hr. There were no toxic symptoms, and no mortality in $CC1_4$ treated control group. AST and ALT activities were also remained unaltered. EChE was regenerated at 4 hr, while PChE was regenerated at 24 hr. Considerable regeneration of ChE activities were evidenced during 6 days.

Chronic studies :

Sublethal doses of malathion (100, 200, and 400 mg/kg) produced mild toxic symptoms which is predominant of muscarinic signs and resolved in all the rats within 4 days. Subsequently rats were symptom free during the rest of the experimental period. Results of the study are shown in Table I. On 14th day of chronic exposure, EChE activities were significantly low ($P<0.001$). On 28th day (14 days after the withdrawal of malathion), ChE, AST, and ALT activities remained unaltered ($P< 0.001$).

In $CC1_4$ treated control group, EChE and PChE activities were significantly low ($P<0.001$), while AST and ALT were significantly ($P<0.001$) increased. The

TABLE I : Enzyme activities during and after malathion exposure in normal and CCl_4 rats. Enzyme activity expressed in U or IU ±SE.

Groups (n=6)	*Chronic exposure (data on 14th day)*				*Recovery after exposure (data on 14th day)*			
Malathion	*EChE U/ml*	*PChE U/ml*	*AST IU/1*	*ALT IU/1*	*EChE U.ml*	*PChE U/ml*	*AST IU/1*	*ALT IU/1*
With out CCl_4								
Control	2.7±0.07	0.3±0.03	8.9±0.3	6.1±0.4	2.6±0.06	0.3 ±0.02	9.0±0.3	6.0±0.3
*100 mg/kg	1.0±0.03	0.1±0.001	20.4±0.8	13.9±0.7	1.2±0.02	0.1 ±0.001	21.5±1.1	11.9±1.9
*200 mg/kg	0.9±0.04	0.1±0.001	29.6±0.8	17.6±1.7	0.9±0.01	0.1 ±0.002	24.1±1.9	11.7±0.7
*400 mg/kg	0.9±0.02	0.1±0.001	43.0±1.1	32.1±1.1	0.9±0.02	0.1 ±0.002	30.8±1.1	12.4±0.7
With CCl_4								
Control	1.6±0.01	0.2±0.002	41.9±1.7	29.1±1.0	1.8±0.02	0.2 ±0.002	38.1±0.3	23.4±0.1
*100 mg/kg	0.8±0.01	0.1±0.001	39.0±1.7	27.8±1.0	0.9±0.03	0.1 ±0.003	30.4±0.5	21.6±0.4
*200 mg/kg	0.7±0.01	0.1±0.001	44.4±0.5	34.5±1.3	0.8±0.02	0.1 ±0.002	35.7±0.3	23.0±0.2
*400 mg/kg	0.7±0.02	0.1±0.001	55.8±1.9	42.6±0.6	0.8±0.02	0.1 ±0.002	38.1±0.4	25.2±0.7

*P<0.001 treatment with or without CCl_4 compared to the control.

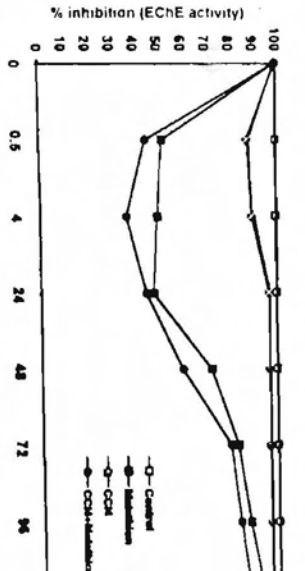

Fig. 1: EChE activity in acute oral exposure malathion in normal and CCl_4 treated rats

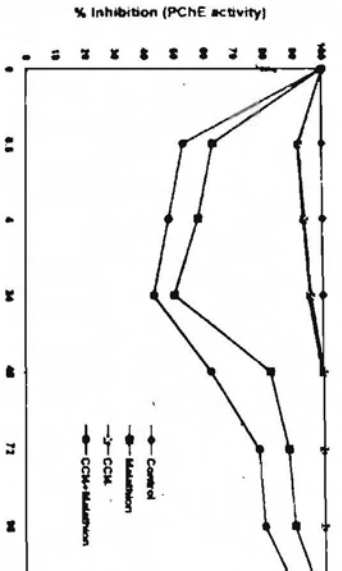

Fig. 2: PChE activity in acute oral exposure malathion in normal and CCl_4 treated rat

enzyme activities remained unaltered e after the withdrawal of malathion for days (Fig. 1 and 2). In CCl_4 treated gr exposed to subletha doses of malathi ChE, AST, and ALT activities were alte significantly (P<0.001). There was significant change after the withdrawal malathion. Histopathological studies sho heptocytes with fatty changes reduction sinusoidal space focal areas of haemorrha and centrilobular necrosis.

DISCUSSION

Acute treatment of CCl_4 did not al the out come of acute exposure of malathi All the rats recovered completely ir shorter period.

Chronic exposure of malathion (25%, 12.5% and 7.25% of LD_{50}) produced only mild symptoms of toxicity for which animals developed tolerance after 3 days. Livers were found to be normal in histopathological examinations. Inspite of it, declined ChE activities did not return to normal even after the withdrawal. All the treated rats exhibited diminished motor activity throughout the treatment period. CCL_4 treated rats exhibited generalised weakness and reduced intake of food. Histopathological examinations revealed liver cell necrosis in CCL_4 trated rats. Concurrent treatment with $CC1_4$ potentiated the chronic toxicity of malathion (Group 8). Liver disorders would probably potentiate the OPC poison. Pre–existing liver disorders due to alcohol, infections, and various drugs such as antitubercular agents, paracetamol etc. are prevalent in many workers in farms and organic chemical industries. If these subjects are exposed to OPC, toxicological effects will be serious.

Assessment of mild toxicity and recovery can not be based on only one or two measurements. Serial measurements along with liver function test will be useful in both acute and chronic exposure. The ChE assay used to monitor liver function after liver transplantation, will also be considered useful in OPC poisoning with the support of AST and ALT values (14).

REFERENCES

1. Pilz W. Methods of enzymatic analysis. (Ed) Bergmeyer HU. Verlag Chemie–Academic Press. 1974; 831–855.
2. Brown SS, Kalow W, Pilz W, Whittaker M, Woronick K. The plasma cholinesterases: a new perspective. *Adv Clin Chem* 1981; 22: 1–123.
3. Hodgson MJ, Parkinson DK. Diagnosis of organophosphate intoxication. *N Eng J Med* 1985; 313: 329.
4. Uete T, Masui K, Miyauchi M. Comparison of substrates for measuring serum choline esterase activity in hepato–biliary disease. *J Clin Chem Clin Biochem* 1985; 23: 669–675.
5. Zimmerman HJ. Hepatic injury caused by therapeutic agents. In: The Liver: Normal and abnormal functions, part A. (Ed) Becker FF. Marcel Dekker, Inc., New York, 1974; 225–302.
6. Venkataraman BV, NagaRani MA, Andrade C, Joseph T. Improved colorimetric method for cholinesterase activity. *Indian J Physiol Pharmacol* 1993; 37: 82–84.
7. Venkataraman BV, NagaRani MA, Andrade C, Joseph T. Correlation of time course of blood cholinesterase activity and toxic manifestations of acute methylparathion in antidote treated rats. *Indian J Physiol Pharmacol* 1994; 38: 214–216.
8. Rubin E, Hutterer F, Popper H. Cell proliferation and fiber formation in chronic carbon tetrachloride intoxication – A morphologic and chemical study. *Am J Pathol* 1963; 42: 715–728.
9. Recnagel RO. Carbon tetrachloride hepatotoxicity: Status quo and future prospects. *Trends Pharmacol Sci* 1983; 4: 129–131.
10. Slater TF. Biochemical studies on liver injury. In: Biochemical mechanism of liver injury. (Ed) Slater TF, New York: Academic Press, 1978; 1–44.
11. Reitman S, Frankel S, Sonenwicth AC. Clinical laboratory methods and diagnosis. The Mosly CV Company, St. Louis. 1970; 1: 82–83.
12. Evans DB, and Lehmann H. Pseudocholinesterase activity in liver transplantation. *Lancet* 1971; 1: 1040–1044.