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Effect of Malathion on Plasma Ache Activity of Mice

VARSHA WANKHADE, K. M. KULKARNI[1] AND A. R. MALU[2]

Department of Zoology, Pune University, Pune, India
[1]Office of Director, Higher Education, Maharastra State, Pune,India
[2]Department of Zoology, Arts and Science college, Aurangabad, India

Abstract

An experiment was conducted to study the effect of malathion on acetylcholinesterase activity in mice plasma. Mature mice were exposed to different doses of malathion for different time period. On dissections, enzymatic estimations were done for each exposure period. It was found that malathion inhibited the acetylcholinesterase activity in mice. The maximum period of exposure was upto 30 days. The degree of inhibition increased upto 4 days of exposure period but declined later on. There was maximum recovery of AChE activity by day 30 of exposure. These findings indicate that the continuous and prolonged exposure to sublethal dose of malathion resulted in the recovery of AChE activity.
Key words : Malathion, *Mus musculus*, Plasma, AChE activity.

Malathion is most widely used organophosphate insecticide throughout the world. It is used to control the pests of agriculture crops, ornamentals, green houses, live stocks, stored grains, forests, buildings and gardens. contributing to its popularity is malathion's low acute mammalian toxicity. But like DDT and other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than it is believed.The toxicity of malathion is compounded by its metabolites and contaminants. Malaoxon, the metabolite produced by the oxidation of malathion in mammals, insects, plants is the primary source of malathion's toxicity and it is 40 times more acutely toxic than malaoxon. Acetyl cholinesterase plays a key role in the control of nerve excitability at post synaptic sites. Malathion is found to inhibit the acetylcholinesterase. Inhibition of plasma acetylcholinesterase (AChE) activity is generally regarded as an useful indicator of poisoning by organophophorous pesticides. Mice have been selected for present study as they have physiological systems and responses similar to those of man. They have also a remarkable genetic similarities to human.

Methods

Only the healthy pairs of mice were were housed in the separate cages. The temperature of house was maintained in the range of 20 to 25 C. The animals were fed on commercially available pellet diet. Mature and healthy mice of either sex weighing between 30 to 40 g were divided into two groups. Animals in each group were maintained on specific diet. The animals of group I were fed a stock diet used as control. Animals from group II were given malathion orally (80.6 mg/kg body weight per day) in a suspension made in distilled water. Mice were selected for sublethal group exposed to only control diet and sacrificed at the end of the experimental period of thirty days (20 mice).

Group II mice were exposed to sublethal concentration of malathion i. e. 1/3 of LC 50/96 h. Group II was further divided into five sub-groups, each of 4 mice depending on the malathion exposure period : GI-2 days, GII-4 days, GIII-8 days, GIV-15 days, GV-30 days.

After the start of experiment, each sub-group at respective treatment period was sacrificed and was used for enzyme analysis. Before this, lethal toxicity tests were carried out for four different concentrations of malathion.

Enzyme Extraction. Pesticide treated mice were killed by cervical dislocation and were dissected. Thorasic cage was opened, the blood withdrawn directly from heart with syring, transferred to heparinized tubes (graduated) and centrifused at 1,000 rpm

Table 1. Effect of malathion on plasma AChE activity of mice Michaelis Menten method. Vmax expressed as A/0. 1 protien/min, km expresssed as mM of AT,ChI, *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$ Parenthetic values are percent change from control.

Exposure period (days)	Vmax ± SD	Km ± SD	Vmax/Km
Control	0.084 ± 0.0032	0.250 ± 0.0025	0.336
2	0.051 ± 0.0031	0.290 ± 0.0039	0.175 (–47.92)
4	0.042* ±0.0042	0.315* ±0.0028	0.133 (–60.42)
8	0.060 ±0.0026	0.300 ±0.0027	0.214 (–36.31)
15	0.071 ± 0.0050	0.280 ±0.0028	0.253 (–24.71)
30	0.077 ±0.0058	0.260 ±0.0034	0.296 (–11.91)

for 10 minutes. The plasma was removed with pipette. Estimation for acetylcholinesterase was done by Ellman method.

Results

Michaelis Menten plots drawn by using data on the effect of various substrate concentration on initial velocity (v) of plasma AChE from control and malathion exposed mice for 2, 4, 8,15 and 30 days of exposure period are shown in Table 1. Maximum decrease in Vmax/Km ratio was observed after 4 days of exposure to pesticide treatment while minimal decrease in the same ratio was observed at the end of 30 days of exposure. Vmax/Km ratio decreased upto 4 days of exposure to malathion treatment, after which gradual uplit in Vmax/Km ratio occured upto 30 days of exposure to malathion.

Discussion

AChE determination of plasma has been used as a good method to evaluate exposure to organophosphoric pesticides. Intoxication by phosphorated pesticides is due to the inhibition of the enzyme acetylcholinesterase in exposed organisms. Plasma acetylcholinesterase levels is useful clinical method to evaluate exposure to organophosphoric pesticide (1).

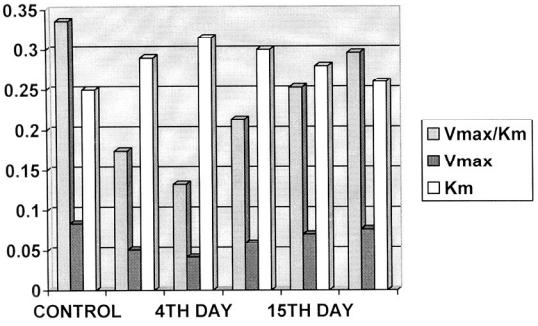

Figure 1. Effect of malathion on plasma AChE activity of mice. Vmax expressed as A/0.1protien/min, Km expressed as mM of ATChI.

Malathion could interact also with DNA of somatic cells as shown by the micronuclei test. Imamura and Talcott (2) have demonstrated *in vitro* the alkylating properties of malathion. It is well known that the inhibition of brain AChE by OP pesticide leads to the accumulation of acetylcholine in the synapses that, in turn, induces hyperactivity of cholinergic pathways. Since acetylcholine plays important trophic roles in brain development, one could assume that OP pesticide exposure during the developmental period can interfere with neurotransmitter function leading to neurodevelopmental abnormalities by disrupting the timing or intensity of neurotrophic actions (3)

Oxidative stress has been postulated to represent a non-cholinergic mechanism by which OP pesticides cause neurotoxicity (4, 5). The relationship between oxidation stress and OP pesticide induced toxicity is still unsolved. There are several contradictory reports (6—10). Divergent results may be related to different OP pesticide compounds and exposure regime employed, which refer mainly to different doses, different times of chronic administration and different ages of the animals at the time of exposure.

It is suggested that a commercial paraquat preparation (a popular herbicide) inhibits cholinesterases with similar or higher potency than classical pesticide inhibitors. Furthermore, this inhibition was observed both in human serum and snake venom, a newly studied source of AChE (II). Plasma AChE was inhibited in chicken after the exposure to malathion. (12).

Results showed that malathin significantly ($P<0.05$) induced free radicals in plasma, liver, testes, brain due to malathion administration. The activity of

acetylcholinesterase was significantly decreased in brain and plasma. The present results suggest that vitamin E seems to have a beneficial effect in alleviating the effects of malathion (13).

Kinetic analysis of the interaction of malathion with camel erythrocyte acetylcholineserase was investigated. The K map and Vmaxapp were both decreased by increased malathion concentration. Dixon and Lineweaver-Burk and their secondary replots indicated that the nature of the inhibition was of pure incompetitive (14).

The magnitude of inhibition in peripheral tissue does not accurately reflect the central inhibitory effect of malathion on AChE activity on specific brain region (15). Statistically significant inhibition by malathion was observed in blood, liver and CNS (16).

The present study revealed that organophosphate pesticide malathion inhibited the acetylcholinesterase in mice brain. The degree of inhibition increased upto 4 days of exposure period. On further exposure to malathion, the AChE inhibition was in following decreasing order : 4 days>8 days>15 days>30 days>.There was recovery of AChE activity by the 30-day of exposure period. These findings indicated that the continuous and prolonged exposure to sublethal dose of malathion resulted in the recovery of AChE activity. Such recovery in enzyme activity might be due to the product degradation or elimination which is yet to be confirmed. The partial recovery of brain AChE exhibited a possible damage in the central and peripheral nervous system. This study indicated that measuring AChE activity in mice plasma is not a sensitive indicator of exposure to malathion. It is necessary to develop more sensitive tests to detect exposure of malathion, which will be helpful to understand the hazardous effects of such widely used pesticide.

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