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Effect of malathion on liver ache activity of mice

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Abstract

An experiment was conducted to study the effect of malathion toxicity on acetylcholinesterase activity in mice liver. 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 liver. 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 30th day of exposure. These findings indicate that the continuous and prolonged exposure to sublethal dose of malathion resulted in the recovery of AChE activity.

Keywords: Malathion, *Mus musculus*, Liver, AChE.

Introduction

Malathion is most widely used organophosphate insecticide through out 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 the product label would lead one to believe. The toxicity of malathion is compounded by its metabolites and contaminants. Malaoxon, the metabolites 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.

Malathion is organophosphorous pesticide extensively used to control a wide range of sucking and chewing pests of field crops, fruits and vegetables. It has many structural similarities with naturally occurring compounds, and their primary target of action in insects is the nervous system; it also inhibit the release of the acetylcholinesterase at the synaptic junction (Cabello et al 2001). Acetylcholinesterase plays a key role in the control of nerve excitability at post synaptic sites. Malathion is found to inhibit the acetylcholinesterase. Inhibition of liver acetylcholinesterase (AChE) activity is generally regarded as a useful indicator of poisoning by

organophosphorous 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.

Materials and Methods

Only the healthy pairs of mice 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 gm 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 a 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 50/96h.

Group II was further divided into five subgroups, each of 4 mice as under; depending on the malathion exposure period.

G1-2days, GII-4days, GIII-8days, GIV-15days, GV-30days.

After the start of experiment, each subgroup 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 the malathion.

Enzyme extraction

Pesticide treated mice were killed by cervical dislocation and were dissected. Liver was taken out, washed with 0.9% NaCl and homogenized in 0.9% NaCl. Estimation for acetylcholinesterase was done by Ellman method (1961).

Statistical analysis

Statistical analyses were performed using analysis of variance (ANOVA), followed by post-

hoc (Bonferroni) least significant difference testing where $P < 0.05$ on ANOVA. Data were presented as mean \pm SD and $P < 0.05$ was considered statistically significant. All calculations were made using SPSS for Windows 10x0 program.

Results

Michaelis Menten plots drawn by using data regarding the effect of varying substrate concentration on initial velocity (v) of liver AChE from control and malathion exposed mice for 2, 4, 8, 15 and 30 days of exposure period are shown in table1.

Table 1: Effect of malathion on liver AChE activity of mice

Exposure period (in days)	Vmax \pm SD	Km \pm SD	Vmax/Km
Control	0.084 \pm 0.0032	0.240 \pm 0.0025	0.278
2 days	0.060 \pm 0.0041	0.410* \pm 0.0018	0.146 (-47.49)
4 days	0.054 \pm 0.0027	0.448 \pm 0.0033	0.120** (-56.84)
8 days	0.075 \pm 0.0037	0.380 \pm 0.0021	0.197 (-29.14)
15 days	0.0082* \pm 0.0037	0.355 \pm 0.0021	0.230 (-17.27)
30 days	0.087 \pm 0.0035	0.340 \pm 0.0018	0.255 (-8.26)

Vmax expressed as A/0.1 protien/min.

Km expressed as mM of ATChI

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$

Parenthetic values are % change from control.

Maximum decrease in Vmax/Km ratio has been observed after 4 days exposure to pesticide treatment while minimal decrease in the same ratio has been observed at the end of 30 days exposure. Vmax/Km ratio decrease upto 4 days of exposure to malathion treatment. After which gradual uplift in Vmax/Km ratio takes place upto 30 days exposure to malathion.

Discussion

Eserine, parathion and malathion are cholinesterase inhibitors responsible for the hydrolysis of body choline esters, including acetylcholine at cholinergic synapses. Acetyl cholinesterase activity decreased in the serum

of rats. The results indicate that organophosphorous pesticides induce changes in the epithelium of mammary gland influencing the process of carcinogenesis, and such alterations occur at the level of nervous system by increasing the cholinergic stimulation (Cabello et al 2001).

Study clearly indicate that malathion used as a commercial product i.e. containing malaaxon and isomalathion, can be considered as a genotoxic substance *in vitro*. Malathion may also produce DNA disturbances *in vivo*, such as DNA breakage at sites of onchogenes or tumour suppressor genes, thus playing a role in the induction of malignancies in individuals exposed to this agent. Therefore, malathion can be

regarded as a potential mutagen/carinogen and requires further investigation (Blasiak at al 1999). In vitro detoxificatio of the organophosphate (OP) insecticides

paraoxon, chlorpyrifosoxon and malaoxon has been investigated in human serum (Sams and Mason1999).

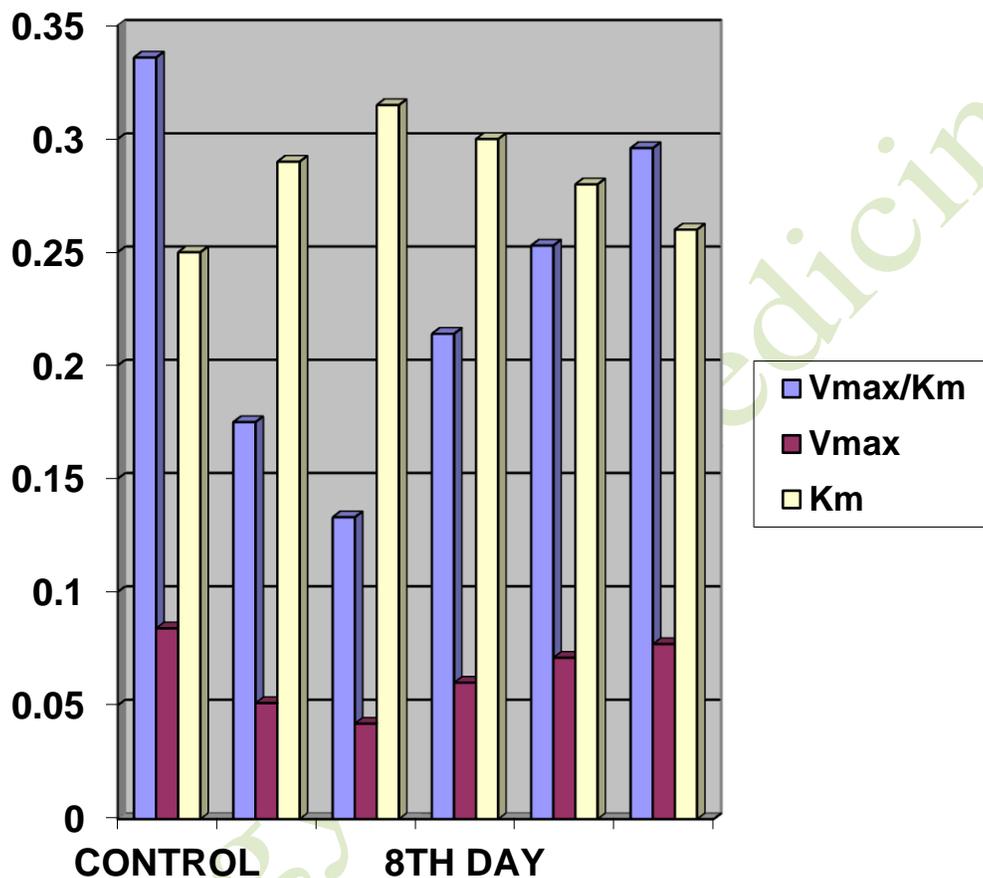


Fig 1: Graphical representation of effect of malathion on liver AChE activity of mice Vmax expressed as A/0.1 protien/min. Km expressed as mM of ATChI

Mineau reported that after exposure to carbamate and organophosphate cholinesterase activity in wild birds decreased (Mineau,1993). Shakoori et al reported 84% inhibition in Tribolium castanem due to sublethal doses of cypermethrin (Shakoori et al 1995). Gard and Hooper reported that organophosphors and carbamate exert their effects by binding to and inhibiting the acetylcholinesterase enzyme at nerve synapses (Gard and Hooper 1995). Organophosphorous compounds may induce

oxidative stress leading to generation of free radicals and alterations in antioxidant and scavengers of oxygen free radicals (OFRs). The effect of subchronic exposure to malathion in the production of oxidative stress was evaluated in male Winstar rats (Maryam Akhgari etal 2003). Administration of malathion (100,316,1000,1500 ppm) for 4 weeks increased catalase (CAT), superoxide dismutase (SOD) activities as well as maalondialdehyde (MDA) concentration in red blood cells (RBC) and liver.

However, acetylcholinesterase (AChE) and cholinesterase (CHE) activities were decreased in these samples. The increase in RBC and liver peroxidation correlated well with the inhibition in RBC AChE and liver CHE activities (Maryam Akhgari et al 2003). Azmi et al (1999) studies the effects of tetranortriterpenoids (Neem product SDS) and deltamethrin (pyrethroid) on phosphomonoesterase activity in *Cyprinus* (common carp) and reported enzyme inhibition under the effect of these pesticide (Azmi et al 1999). The result demonstrate that the magnitude of AChE inhibition in peripheral tissues does not accurately reflect the central inhibitory effects of malathion on AChE activity in specific brain regions (Banasiak 2003). Burgees et al (1999) observed that a organophosphate insecticide reduced cholinesterase activity in birds (Burgees 1999). Taylor et al reported that a sublethal dose of field grade malathion (0.01 mg/g) lowered brain cholinesterase levels by 22% and 175, respectively (Taylor et al 1999). Parson et al observed effect of organophosphate and carbamate on non target wild animals and these pesticides inhibited cholinesterase activity (Parson et al 2000). The potential use of acetylcholinesterase (AChE) and metallothionein (MT) responses as biomarker of organophosphorous (OPs) and trace metal were assessed in fish *Seriola dumerilli* brain AChE was significantly inhibited after 2 nd 7 days of malathion exposure (Jebali, 2006). Khan studied the effect of permethrin and biosal in the Indian garden lizard and reported that after treatment with permethrin cholinesterase levels decreased by up to 17% and 19% in the kidney and 18% and 24% in the liver (Khan et al, 2002). The study indicates that malathion inhibited the AChE activity in brain of mice (Wankhade et al 2008). In present study, we also found the decrease in the activity of acetylcholinesterase after the exposure of mice to malathion. Activity of acetylcholinesterase was recovered later.

Conclusion

Michaelis-Menten plot drawn by using data regarding the effect of various substrate concentrations on initial velocity (v) of liver 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 V_{max}/K_m ratio was observed after 4 days of exposure period to malathion treatment while minimum decrease in the same ratio was observed at the end of 30 days of exposure period. V_{max}/K_m ratio decreased upto four

days of exposure to malathion. After which gradual uplift in V_{max}/K_m ratio took place up to 30 days of exposure to malathion.

These findings indicates that the continuous and prolonged exposure to sublethal dose of malathion resulted in the recovery of AChE activity.

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