THE EFFECT OF MALATHION DUST ON CERTAIN TISSUES OF MALE RATS FED VARYING LEVELS OF DIETARY PROTEIN

I. VAISHWANAR AND S. MALLIK

Department of Biochemistry,
Government Medical College, Nagpur - 440 003

( Received on August 4, 1983 )

Summary: The effect of malathion, an organophosphorus insecticide on tissue levels of acetylcholinesterase (Ache), phosphomonoesterases and transaminases have been studied in presence of different levels of dietary proteins. Adult male albino rats weighing 150-200 gms were given 5%, 10% and 20% protein diets containing 400 mg malathion (dust) 5% conc./kg feed for 30 days. Its effect was evaluated in liver, kidney, brain, lungs and spleen and results were compared with their respective malathion dust, pair-fed animals (5%, 10% and 20% protein groups without malathion).

Animals kept on low protein diets (5% and 10%) when exposed to malathion dust showed significant increase in the activities of GOT and alkaline phosphatase in liver, kidney, brain, lungs and spleen, while a marked inhibition in the activity of Ache was observed under similar treatment. GPT was decreased in kidney and lungs, in the low protein groups (5% and 10%), whereas its activity was increased in liver, brain and spleen of animals receiving 5% protein, when exposed to compared to their respective pair-fed animals.

Thus, although the degree of alteration in the enzyme profile is less severe, these changes show that high protein diet has a protective role against pesticide hazards, whereas low protein diet provides less stability to the structural integrity of the tissues.

Key words: GOT-glutamic oxaloacetic transaminase GPT-glutamic pyruvic transaminase Ache-acetyl cholinesterase

INTRODUCTION

Organophosphorus pesticides are finding wide application for the protection of the crops since it is considered to be a safe pesticide for human beings and higher animals. However, the report of FAO/WHO 1972 (14) show that some residual amounts of these pesticides are present in the various food stuffs and drinking water.

The toxicity of these agents are correlated with dietary protein levels. Boyd and Chen (6) in a study of lindane toxicity and protein deficient diet found that lindane was twice as toxic when given to male albino rats fed a diet low in protein. Boyd and Krijnen (8) further suggested that the ability to detoxify pesticides might be impaired
in animals malnourished from the feeding of a protein deficient diet. Further Boyd et al. (7) studied endosulfan toxicity with various dietary protein levels (0%, 3.5%, 9%, 26%, 81%) and found similar results. In another experiment, Boyd (5) found that malathion was 2–3 times more toxic in animals fed a diet containing 35% protein (low as casein than in animals fed a normal protein casein diet (26%). Malathion, an organophosphorus insecticide is used most commonly in our country. Therefore, malnourished population, as is quite prevalent in our country are likely to be susceptible to the various kinds of health hazards.

Various workers have tried different doses of malathion and observed that 1375 mg/kg body weight is lethal (15), 600–900 mg/kg body weight is highly toxic (24), mild toxicity was seen with 300 mg/kg (4), and significant change with 400 mg/kg dose (31).

In view of the above available literature, it was considered to be worthwhile to study the effect of 400 ppm malathion on the susceptibility of lab animals under controlled conditions, to assess whether protein malnutrition dose, in fact potentiate the systemic action of malathion, and to assess whether dietary protein could ameliorate the adverse effect of pesticide in vivo. This would provide valuable clues for diet formulation for the exposed population.

**MATERIAL AND METHOD**

Male albino rats weighing approximately 150–200 g were divided into three experimental groups of ten rats each. They were matched with their respective controls of 10 rats each (i.e. pair-fed animals). The animals were fed *ad libitum* with the purified diet comprising of crude fiber 1%, fat 8%, vitamin mixture 1%, mineral mixture 5%, starch to make up the volume to 100 g and moisture 5%. The experimental rats were given oral daily dose of 400 ppm malathion dust (5% conc.) through diet and maintained on 5%, 10% and 20% casein protein diet respectively.

After one month of pesticide exposure the animals were killed by decapitation. Brain, liver, kidney, lungs, and spleen were removed, immediately washed with ice cold saline and chilled to 0°C in an ice-bath. The tissues were homogenized and diluted with ice cold normal saline solution.

The enzyme acetylcholinesterase was determined as described by Hestrin (19). All the other enzymes, aspartate transaminase, alanine transaminase and alkaline phosphatase were estimated as described by King and Wootton (31). Protein percentage was estimated by the method of Lowry *et al.* (28).
RESULTS

Table I shows that the GOT activity in liver, kidney, brain, lungs, and spleen was significantly ($P<0.001$) increased in rats fed malathion in presence of 5% dietary protein than in their respective pair-fed control animals (5% protein only). Malathion treatment to 10% dietary protein group increased GOT activity significantly in liver ($P<0.001$) and brain ($P<0.002$), whereas, decreased in kidney ($P<0.005$) and lungs ($P<0.05$) with no significant effect on spleen GPT activity, when compared to the pair-fed controls (10% protein group). There was no significant difference in the GOT activity in malathion treated rats fed 20% dietary protein as compared to their pair-fed controls (20% protein only). As shewn in Table I, oral exposure of rats to malathion at 5% dietary protein level resulted in a significant increase in liver ($P<0.001$), brain ($P<0.001$) and spleen ($P<0.001$) GPT activity, however the activity was decreased in kidney ($P<0.001$) and lungs ($P<0.001$) than their pair-fed controls. In the 10% dietary protein group only liver ($P<0.05$) and lungs ($P<0.01$) showed significant decrease in GPT activity than the 10% control group, whereas in kidney, brain and spleen, it remained unchanged. No significant difference was found in GPT activity of tissues of malathion treated rats of 20% dietary protein as compared to their pair-fed controls.

**TABLE I**: Activity of Glutamic Oxaloacetic transaminase and glutamic Pyruvic transaminase enzymes in Rats fed varying casein diet with malathion dust.

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Glutamic oxaloacetic transaminase (n mole/Pyruvic acid formed/g tissue/min.)</th>
<th>Glutamic Pyruvic transaminase (nmole acid formed/g tissue/min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>5% Protein</td>
<td>4.5</td>
<td>4.28</td>
</tr>
<tr>
<td>Control (10)</td>
<td>±0.04</td>
<td>±0.01</td>
</tr>
<tr>
<td>5% Protein</td>
<td>5.84**</td>
<td>4.74**</td>
</tr>
<tr>
<td>Malathion (10)</td>
<td>±0.03</td>
<td>±0.02</td>
</tr>
<tr>
<td>10% Protein</td>
<td>0.905</td>
<td>1.515**</td>
</tr>
<tr>
<td>Control (10)</td>
<td>±0.10</td>
<td>±0.06</td>
</tr>
<tr>
<td>10% Protein</td>
<td>1.066**</td>
<td>1.218**</td>
</tr>
<tr>
<td>Malathion (10)</td>
<td>±0.1</td>
<td>±0.06</td>
</tr>
<tr>
<td>20% Protein</td>
<td>1.170</td>
<td>3.0</td>
</tr>
<tr>
<td>Control (10)</td>
<td>±0.01</td>
<td>±0.20</td>
</tr>
<tr>
<td>20% Protein</td>
<td>1.177</td>
<td>3.2</td>
</tr>
<tr>
<td>Malathion (10)</td>
<td>±0.01</td>
<td>±0.25</td>
</tr>
</tbody>
</table>

*** $P<0.002$, student's $t$-test,

** $P<0.001$, student's $t$-test,

* $P<0.05$, student's $t$-test.

The figures in parenthesis indicate no. of rats taken in each group.
Malathion treatment for 1 month to the 5% dietary protein group induced significant increase (Table II) on the alkaline phosphatase activity in liver \((P<0.001)\), kidney \((P<0.001)\) and brain \((P<0.001)\) as compared to their respective pair-fed control. In 10% dietary protein group alkaline phosphatase was significantly decreased \((P<0.001)\) in kidney, whereas it remained unchaned in other tissues. The activity of the enzyme Acetyl cholinesterase was (Table II) markedly inhibited \((P<0.001)\) in the liver, kidney and brain tissues of rats fed 5% dietary protein as compared to their controls. The 10% protein diet resulted in the inhibition of AchE in kidney \((P<0.001)\) only, whereas in liver and brain it remained non-significant.

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Alkaline phosphatase (mg Pi liberated/g tissue/min)</th>
<th>Acetylcholinesterase (mole Acetylcholine hydrolyzed/g tissue per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>5% Protein control (10)</td>
<td>0.292±0.02</td>
<td>1.41±0.01</td>
</tr>
<tr>
<td>5% Protein malathion (10)</td>
<td>0.465±0.01</td>
<td>1.68±0.03</td>
</tr>
<tr>
<td>10% Protein control (10)</td>
<td>0.156±0.001</td>
<td>2.088±0.001</td>
</tr>
<tr>
<td>10% Protein malathion (10)</td>
<td>0.150±0.001</td>
<td>2.064±0.003</td>
</tr>
<tr>
<td>20% Protein control (10)</td>
<td>0.189±0.001</td>
<td>2.75±0.31</td>
</tr>
<tr>
<td>20% Protein malathion (10)</td>
<td>0.185±0.001</td>
<td>2.8±0.51</td>
</tr>
</tbody>
</table>

** \(P<0.001\), student's t-test.

The figures in parenthesis indicate no. of rats taken in each group.

**DISCUSSION**

Malathion is considered to be one of the pesticide having anticholinesterase activity. Kurtz (26) observed that doses as high as 100 and 150 \(mg/kg\) of Technical grade malathion produced significant decrease on blood and brain cholinesterase activity, whereas doses as low as 50 \(mg/kg\) failed to exhibit any effect on its activity. Male
corturnix quail (corturnix coturnix japonica) birds when fed graded doses of malathion showed decreased plasma cholinesterase activity (12). Bhatnagar and Malviya (5) similarly reported that oral administration of malathion (687.5 mg/kg body wt.) to adult male rats fasted for sixteen hr showed casual inhibition of serum cholinesterase activity. However, such anticholinesterase activity is reported to be reduced, if sufficient amount of dietary protein is supplied in the diet of the animals (10). Weatherholtz (37) and Lee et al. (27) observed the effect of organophosphorus pesticides (banol, parathion, chlordane, heptachlor, dieldrin) administration upon esterases activities in serum and tissue esterases of rats fed variable casein diets for 30 and 60 days found that protein content of the diet had a significant role on the effect of pesticide toxicity.

The results of the present series of investigation are in agreement with the reports of Casterline et al. (10) that rats on the low protein diet are more susceptible to the exposure of pesticide poisoning than those at higher protein level. The lower the dietary protein level the greater is the degree of cholinesterase inhibiting capacity of malathion.

Alterations in plasma enzyme activities are a manifestation of pathological changes in several organs or tissues (18). Cellular necrosis and fatty tissue changes, particularly in the liver, kidney, cardiac and skeletal muscles are associated with leakage of tissue enzyme into the blood (1, 20, 13.). A number of pesticides including organochlorines, organophosphorus, carbamates and environmental pollutants have been shown to alter the activities in the blood of such enzymes as aspartate aminotransferase 0 (29, 17, 25) cholinesterase (36, 11) and alkaline phosphatase (16). Vos et al. (35) studied the toxicity of hexachlorobenzene in birds and found that 20 ppm and 120 ppm in the diet for 4 weeks increased the activity of aspartate aminotransferase in the serum. Sasinovich and Voronina (34) have shown that organophosphorus and carbamate insecticides increased the activity of aspartate and alanine aminotransferase in serum (12). Therefore, the increased activity of GOT and GPT in rats in our series of experiment can be attributed to malathion exposure. This increase in the activity of transaminase was significant only in the low protein groups, while that of high protein group (20) remained unchanged upon malathion treatment. This indicates that normal or high protein diet resists the adverse effects of pesticides on the tissues of mammalians, especially on the liver.

Bhandari (27) reported increased alkaline phosphatase activity in Rats after 1,2 and 3 hrs of malathion (50 mg/kg) administration, although statistically the change was not significant. Saigal and Malviya (33) observed induction in the alkaline phosphatase activity in male albino rats treated with malathion (687.5 mg/kg body wt.). Our findings on the activity of alkaline phosphatase following malathion dust exposure is in agreement with the reports of above authors. Malathion exposure to animals receiving high protein diets failed to develop much toxic effect on the activity of the enzyme. The increased activity of the enzyme is associated with the malfunctioning of the liver (30).

Albino rats exposed to 400 ppm malathion dust for 1 month showed significant
difference in the activity of the enzymes GOT, GPT, alkaline phosphatase and Ache at 5% and 10% dietary protein level, although the magnitude of alteration in the enzyme profile was marginal. The rats fed 20% dietary protein showed no difference in the activity of all the above enzymes upon treatment with 400 ppm malathion.

The enhanced susceptibility of the low protein groups to the toxicity of malathion could be due to one or more of several factors. The reduction or absence of dietary protein might inhibit protein-insecticide combination in the intestinal lumen and thus-favour the more rapid absorption of the pesticide (7). Secondly, as suggested by Boyd and Krijnen (8), a protein deficient diet could selectively inhibit production of enzymes concerned with the detoxification of the pesticide. Thirdly, a protein-deficient diet might lessen the available number of amino and sulfhydryl groups in cells of the end organs where malathion reacts to produce toxic effects so that a small dose of malathion could react with sufficient percentage of the lowered number of such groups and cause death. Thus the pesticide protein interaction is responsible for the degree of toxicity of malathion.

ACKNOWLEDGEMENTS

The financial assistance by the Council of Scientific and Industrial Research for the research, and Research Fellowship to S. Mallik is gratefully acknowledged. The authors are thankful to Dr. B.R. Solanky for his constant encouragement throughout the work.

REFERENCES


