

Development of Tolerance to Monocrotophos

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Abstract

The tolerance phenomenon after repeated exposure to monocrotophos (MCP) was studied in rats. Rats were daily injected intraperitoneally with either MCP (3 mg/kg) or distilled water (5 ml/kg) for 14 days duration. Mean body weight (MBW) of treated rats declined significantly after 2 days of injections, became stabilized from day 7 to day 10 and later increased with the same rate of growth compared to the control. Tremor and convulsion were most severe during the first 5 days and dropped to below 50 percent of population after 1 week. After 10 days, no animal showed any significant tremor and convulsion. Mortality rates as shown by the percentage of death rate in the population showed two prominent peaks. The first peak of 20 percent of population occurred after 2 days of injection of MCP, and the second peak of 14 percent on day 5. After 8 days there was no more death in the treated group. None of the animals in the control group died or had abnormal cholinergic signs. Twenty-four hours after 1, 3, 7 and 14 injections, rats were decapitated and specific activity of acetylcholinesterase (AChE) in the cerebral cortex, hippocampus and striatum was measured. Specific activity of AChE returned to about 70 percent of control at day 1 but dropped significantly at day 3 and day 7 to 45-60 percent and 30-40 percent of control, respectively, then stabilized throughout the period of the experiment. The experimental evidence suggested that tolerance develops in rats after repeated exposure to monocrotophos.

Key word: Monocrotophos, Acetylcholinesterase, Tolerance, Organophosphate, Brain, Rat.

1. Introduction

It has been demonstrated that a tolerance to the toxicity of some organophosphates (OPs) can be induced by repeated exposure. That is, the typical signs of their toxicity that are seen with initial doses are diminished despite continued dosing and consistent depression of AChE activity [1-3]. Daily treatment with small doses of OMPA (Octamethylpyrophosphorictate-tramide) rendered rats resistant to the lethal affect of larger doses of OMPA [4]. Many investigators have suggested that the phenomenon may result from a decreased sensitivity of muscarinic receptors in response to increased level of ACh [5-7]. Nowadays, there are many experimental

tests of hypothesis about the neuronal mechanisms underlying behavioral tolerance to some OPs. The previous studies were designed to test predictions from the following four major categories of hypotheses: 1) a decreased sensitivity of muscarinic receptors [6-7], 2) nonspecific metabolic changes in postsynaptic neuron [8], 3) the reduction in de novo synthesis of acetylcholine (ACh) [9], 4) the shunting of activity into another pathway e.g. adrenergic, GABAergic [10-11].

The study presented here was undertaken to investigate the tolerance phenomenon after repeated sublethal exposure to monocrotophos (MCP), a widely used OP insecticide by measuring several parameters including mean

body weight, cholinergic signs such as tremor and convulsion, mortality and corresponding levels of AChE activity.

2. Materials and Methods

Adult male Wistar strain rats approximately 180-220 g weight were used. Animals were housed and maintained in room with a 12 hour light/dark cycle and provided with diet and water ad libitum.

MCP purity was verified by HPLC technique at Agricultural Toxic Substance Division, Department of Agriculture. MCP was dissolved in distilled water just before use, and the injection volume for both treated and control groups was 5 ml/kg body weight.

Rats were injected intraperitoneally daily (at 10.00-11.00 am.) with either MCP (3 mg/kg) or distilled water (5 ml/kg) for 14 days duration. Body weight of rats was recorded before each injection. Abnormal cholinergic signs were observed daily in the all or none fashion (i.e. whether they are present or absent) and separately scored on individually isolated rats as 0 = absent and 1 = present. Mortality rate was recorded within 24 hrs for each injection. Animals were decapitated 24 hrs after 1,3,7 and 14 daily doses. The cerebral cortex, hippocampus and striatum were dissected out, specific activity of AChE and protein concentration were determined.

Determination of AChE activity

Tissues were homogenized in sodium phosphate buffer (0.1 M pH 7.4) at a concentration of 40 mg wet weight per ml buffer. The homogenate was centrifuged at 9000 g for 5 min and used as the enzyme source. AChE activity was measured by a slight modification of the method of Ellman et al., [12], with acetylcholine iodide (0.75 M Sigma Chemical Co.) as substrate.

Protein determination

Protein concentrations were measured by the method of Lowry et al., [13] using Bovine Serum Albumin as standard.

Statistical analysis

Results were expressed as group mean \pm SEM. Statistical comparisons of group means were made by Student's t-test and linear

correlation coefficient. The minimum acceptable level of significance was 0.05.

3. Results

The effect of repeated exposure to MCP on cholinergic signs, MBW and mortality

Rats were treated with either MCP (3mg/kg/day or approximately 0.6 LD₅₀) or distilled water (5 ml/kg/day) via intraperitoneal injections for 14 days duration. Severe signs of cholinergic toxicity were observed in the treated group. The onset of abnormal cholinergic signs was within 15 to 30 minutes and diminished within 2 hrs. Tremor, convulsion and fasciculation were minimal or absent within 4 hrs after the injection. Tremor and convulsion became more severe during the first 5 days and dropped to below 50 percent of the population after 1 week. After 10 days, no animal showed any significant tremor or convulsion.

Mortality in the treated group was observed exclusively during the first 2 hrs after each injection. Mortality rate as shown by the percentage of dead rats in the tested population showed two prominent peaks. The first peak of 20 percent of population occurred after 2 days of injection of MCP, and the second peak of 14 percent on day 5. After 8 days, there were no more deaths in the treated group.

None of the animals in the control group died or had abnormal cholinergic signs.

Body weight was recorded before each injection, MBW of treated rats declined significantly ($p < 0.025$) after 2 days of injection and became stabilized from day 7 to day 10 and increased as dosing continued although treated rats did not reach body weight attained by control rats.

The effect of repeated exposure to MCP on AChE activity in rat brain regions

Specific activity of AChE in the cerebral cortex, hippocampus and striatum of rat brain was measured 24 hrs after 1,3,7 and 14 daily dose of either MCP (3 mg/kg/day) or distilled water (5 ml/kg/day) via intraperitoneal injection. Specific activity of enzyme AChE returned to about 70 percent ($p < 0.01$) of the control group after one day but dropped significantly ($p < 0.001$) by day 3 and day 7 to 45 to 60 percent and 30 to 45 percent, respectively and became stabilized throughout the period of

the experiment. The MCP administration produced a significant ($p < 0.001$) reduction of specific activity of AChE in each brain region studied at all time points when compared to control values. The reduction of specific activity of AChE in all brain regions studied at all time points correlated highly

(correlation coefficient values were between 0.977 to 0.999). Specific activity of AChE in striatum at day 7 was significantly different ($p < 0.05$) when compared to day 14.

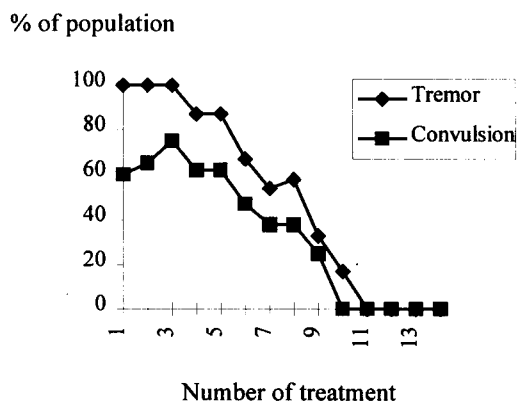


Figure 1. The reduction in the percentage of tremor and convulsion of rats following daily intraperitoneal injection of MCP (3mg/kg/day) for 14 days duration. Values are expressed as percent of population from 12-20 animals. None of the animals in control group had abnormal cholinergic signs

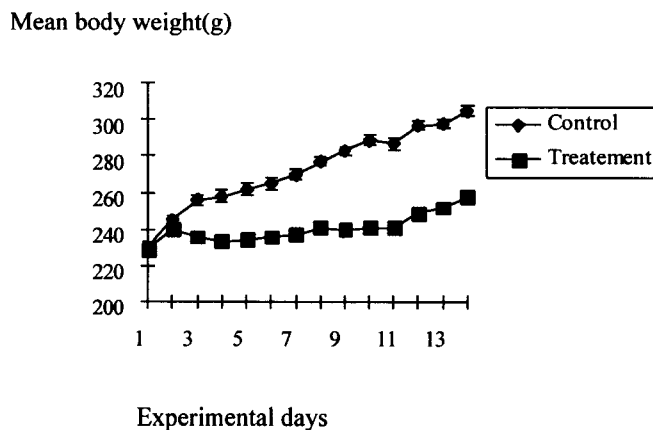


Figure 2. Effects of repeated exposure to MCP (3 mg/kg/day) or distilled water (5ml/kg/day) via intraperitoneal injections for 14 days duration on body weight. Body weight of rats was recorded before each injection. Values are mean \pm SEM from 10-20 animals. a indicates significant difference from day 1. ($p < 0.025$), b indicates significant difference from day 1. ($p < 0.005$), and c indicates significant difference from day 1. ($p < 0.001$).

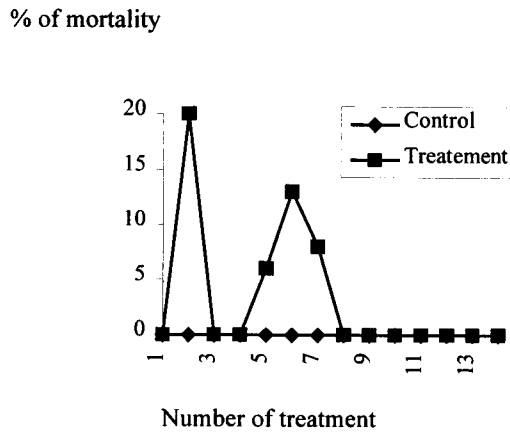


Figure 3. Effects of repeated exposure to either MCP (3mg/kg/day) or distilled water (5ml/kg/day) via intraperitoneal injections for 14 days duration on the mortality rate of rats. Mortality was recorded 24 hrs after each injection. Values are percent of population from 10-20 animals.

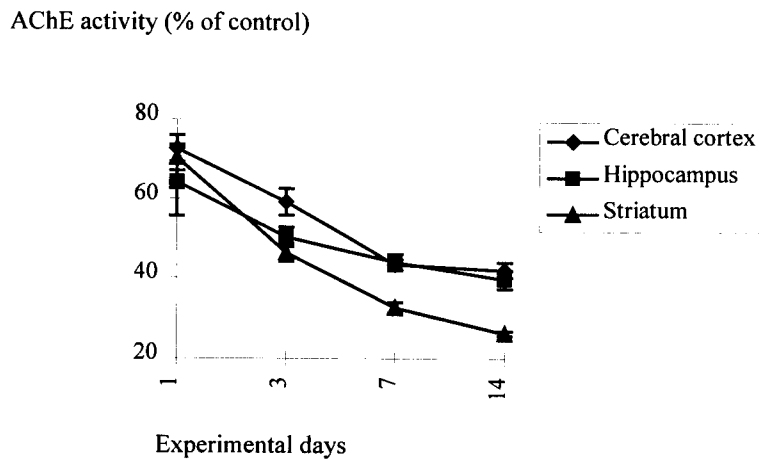


Figure 4. Effects of daily injection of MCP (3 mg/kg/day) or distilled water (5ml/kg/day) via intraperitoneal injections for 14 days duration. Specific activity of AChE versus times in the cerebral cortex, hippocampus and striatum are expressed as mean±SEM from 8 animals in each group. All specific activities of AChE presented are significantly different (a, $p < 0.001$) from control animals as determined by Student's t-test. Specific activity of AChE in the striatum at day 7 was significantly different (b, $p < 0.05$, by Student's t-test) when compared to that of day 14.

4. Discussion

Previous experiments have shown that a tolerance to the toxicity of OP compound including DFP, malathion and tetram developed during repetitive exposure to sublethal doses. In addition to these findings, some investigators also demonstrated that MBW of malathion-treated mice initially declined rapidly, but by day 5 had stabilized, and subsequently growth rates seemed to be normal.

In this study, several parameters including mean body weight (MBW), tremor, convulsion, mortality and specific activity of AChE in the cerebral cortex, hippocampus and striatum were measured daily in adult male Wistar strain rats after intraperitoneal injection of MCP 3mg/kg/day for 14 days duration. MBW declined significantly after 2 days of injection but became stabilized from day 7 to day 10 and increased as dosing continued. However, treated rats did not reach weights attained by the control group. Tremor and convulsion became more severe during the first 5 days and dropped to below 50 percent of the population after 1 week. After 19 days, no animals showed any significant tremor and convulsion. Mortality rate as shown by the percentage of death rate to the population showed two prominent peaks. The first peak of 20 percent of population occurred after 2 days of injection of MCP, and the second peak of 14 percent on day 5. After 8 days, there were no more deaths in the treated group. This result indicated that rats become tolerant to MCP, after repeated exposure and this generally supports previous literature reports of OP tolerance. In contrast to this study, Fernando et al. [14] showed that there was lack of tolerance of rats to repeated of the GABA system may be a part of a compensatory inhibitory process to counteract the excessive cholinergic activity produced by MCP.

From previous studies, the AChE activity was inhibited to the greatest degree in the cerebral cortex, hippocampus and striatum and least inhibited in the hypothalamus after treatment with some OPs such as DFP and tetram [19]. This study reported that daily intraperitoneal injection of MCP (3mg/kg/day) for 14 days duration caused significant

exposure to soman and sarin. Repeated treatment with soman (90 µg/kg) and sarin (100µg/kg) at 4 day intervals caused increasing mortalities and variable incidences of tremor and convulsion and body weights of treated rats were slightly but not significantly lower on successive treatment days. They proposed that the profile of toxicity of these OPs should be different from, but not generalized with that of the other OPs. In contrast, the present result also indicated that development of tolerance was produced after repeated exposure to sublethal dose of MCP which induced convulsion. Furthermore, the two peaks of relatively higher death rates indicate that there were multiple processes and factors which underline tolerance to MCP. One may be a rapid process of recovery in AChE from MCP inhibition which is responsible for the early drop in death rate. The second peak of high death rate found in the present study may be due to a second redistribution of MCP from other compartments in the body before a long-term process of tolerance can be initiated most probably by either plastic changes in ACh receptors and/or de novo synthesis of AChE. It is possible that GABAergic system may be involved in this phenomenon, since previous experiments have shown that γ -amino butyric acid increased significantly after repeated exposure to MCP and Lundy and Magor [16] and Lundy et al. [17] showed that small amounts of benzodiazepines, which are believed to act through enhancing GABAergic transmission [18] totally abolished OP-induced convulsions, whereas the antimuscarinic agent atropine had no effect, even in high doses. Therefore the involvement ($p < 0.001$) reduction of specific activity of AChE in the cerebral cortex, hippocampus and striatum when compared to control groups. Specific activity of enzyme AChE returned to about 70 percent of control after one day but dropped significantly ($p < 0.001$) by day 3 and by day 7 when compared to day 1 to day 45 to 60 percent and 30 to 45 percent of control, respectively and became stabilized throughout the rest of the experiment in all brain areas. In addition, Bartholomew et al, [7] demonstrated that tolerance induced by repeated exposure to

malathion is cellular type and not a metabolic type because the MFO activity was not induced and the reduction of AChE activity was continuous throughout period of dosing. So, the present result was compatible to the finding which was obtained by Bartholomew et al. [7].

Nowadays, there are many possibilities for the experimental test of these hypotheses on the neuronal mechanisms underlying behavioral tolerance to some OPs. The previous studies were designed to test predictions from the following four major categories of hypotheses: a) a decreased sensitivity of muscarinic receptor in response to increased level of AChE [5-7], b) nonspecific metabolic changes [8], c) end-product inhibition [9] and d) a shunting of activity into another pathway [10-11].

Until now, the mechanism underlying tolerance to OPs is still a controversy and needs further studies.

5. References

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