CORRELATION OF TIME COURSE OF BLOOD CHOLINESTERASE ACTIVITY AND TOXIC MANIFESTATIONS OF ACUTE METHYLPARATHION IN ANTIDOTE TREATED RATS

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Abstract: Study was conducted to find out the correlation between red blood cholinesterase (RBC ChE) and plasma butyryl cholinesterase (BuChE) activities and toxic signs of oral methylparathion (MPT) and their recovery pattern with or without atropine treatment in female rats. Enzyme activity was estimated before and after an oral dose of MPT (7.5 mg/kg⁻¹) at various time intervals up to 120 hr. Antidote groups received atropine (10 mg/kg⁻¹, i.p.), either alone or with diazepam (2.5 mg/kg⁻¹, i.p.), at the onset of toxic signs. Inhibition of enzyme activity served as a definite index of acute toxicity of MPT. RBC ChE activity correlated with the intensity of toxic signs in no-antidote rats, while in atropine treated groups, there was no correlation. BuChE levels did not correlate with toxic signs in any of the groups except in the fatal group. The resynthesis of both the enzymes was complete in 120 hr study and did not synchronize with the recovery pattern of animals from toxic signs. Compared to BuChE, RBC ChE activity was found to be a more sensitive indicator for the diagnosis of severity of MPT toxicity.

Key words: methyl parathion RBC cholinesterase
cholinesterase butyryl atropine diazepam
to simulate the treatment of acute poisoning in humans. Aim of the study is to find out the longitudinal pattern of inhibition and regeneration of RBC ChE and plasma BuChE activities at various time intervals and to relate them with toxic manifestations.

Oximes are not included in this study as they interfere with the enzyme estimations and in their absence, the regeneration of ChE activity depends on de novo synthesis.

METHODS

Adult female Wistar rats weighing 170 g with free access to food and water ad lib were used. In preliminary
studies, LD$_{50}$ of MPT (7.5 mg/kg$^{-1}$) was determined. The stock solution of MPT (50% w/w methylparathion, 10% emulsifier and rest solvents, Makam Agrochem Ltd, Bangalore) was preserved for not more than a week in a refrigerator at 4°C and fresh dilution was made each time on the day of the experiment.

**Toxicity studies:**

Rats were randomised into 4 groups and were administered drugs as below.

- **Group 1:** Control - normal saline (0.9%) orally.
- **Group 2:** MPT - 7.5 mg kg$^{-1}$ orally.
- **Group 3:** MPT (as in Group 2) + atropine (10 mg kg$^{-1}$, Sigma) i.p. at the onset of early toxic signs (salivation).
- **Group 4:** MPT (as in Group 2) + atropine (as in Group 3) with diazepam (2.5 mg kg$^{-1}$, Ranbaxy) i.p. at the onset of early toxic signs.

After oral administration of MPT, all the rats were placed in individual trays (35 x 25 x 5 cm) for observation of toxic signs and were graded as follows to indicate the severity of toxicity.

- **Grade I:** Mild: salivation, lachrymation and bronchial secretions (Muscarinic manifestations).
- **Grade II:** Moderate: Fasciculations, tremor and jerks (Nicotinic manifestations).
- **Grade III:** Severe: Convulsions, respiratory paralysis and death (CNS manifestation).

Blood (45 µl) was drawn from the orbital sinus of rats before and $1/2$, 4, 24, 48, 72, 96 and 120 hr after the administration of MPT. RBC ChE activity (4) and BuChE activity (using butyrylthiocholine iodide as substrate) were measured spectrophotometrically (5).

Two way and three way repeated measures ANOVAs were performed to ascertain the changes in ChE activities across time in the 4 groups. Tukey’s Significant Difference post hoc test was used to effect multiple comparisons to localise significant differences identified by the ANOVAs.

**RESULTS AND DISCUSSION**

Rats, in group 2-4, developed toxic signs within 3 min of administration of MPT. Group 2, which did not receive any antidote, showed 36% of mortality in 15-20 min with grade III toxic signs. The rats survived in that group exhibited grade II toxic signs. There was no mortality in the antidote treated rats (group 3, 4) and the toxic signs were confined to grade I. Rats in group 4 did not exhibit any post-toxic depression as seen in group 2-3. All the rats (group 2-4) recovered from toxic signs within $2\frac{1}{2}$ hr and their ChE activities came to baseline values on 6th day. MPT had significantly altered (Tukey’s test, P < 0.05) both the ChE activities in the treated rats (group 2-4) as compared with the control rats (Fig. 1 and 2). The regeneration of RBC ChE was observed after a fall in activity between $1/2$-4 hr while BuChE activity rose steadily after a prolonged decline in the activity between 4-24 hr.

![Butyrylcholinesterase (BuChE) activity in MPT and antidote treated rats](image)

**Fig. 1:** Butyrylcholinesterase (BuChE) activity is MPT and antidote treated rats.

In general, duration of toxic manifestations of most OP compounds is relatively short so that for biochemical studies of acute LD$_{50}$, 2-10 days observation LD$_{50}$ is adequate (1). The inhibited ChEs undergo substantial spontaneous reactivation within one day which facilitates recovery from intoxication and considerable regeneration of ChE activities were
These differences might be due to enzyme specificity differences in access of the compounds to the targets or due to mixture of AChE and BuChE activity in rat serum (1, 6).

Atropine antagonises many of the peripheral muscarinic effects of excess of ACh and also some central effects. Anticonvulsants often complement atropine or atropine + oxime therapy. In particular, addition of diazepam in the treatment regime is known to raise LD$_{50}$ and hasten speed of recovery (2, 7). Similarly in our study, the group which received diazepam along with atropine showed only grade I toxic signs without post-toxic depression. But, diazepam alone is ineffective except in combination with atropine. Persistence of the toxic agent which is released into circulation over a period of few days from fat depots and other organs may delay recovery (8). Thus, single doses of atropine + diazepam are of only marginal efficacy compared to the repeated doses at intervals (9). Comparatively, RBC ChE activity is a more sensitive index for the diagnosis of toxicity of MPT and also for assessment of regeneration of enzyme activity.

REFERENCES


