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# Rat Brain Acetylcholinesterase Response to Monocrotophos and Abate

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The exposure to organophosphorus (OP) pesticides lead to cholinesterase inhibition and cause an accumulation of acetylcholine in the central nervous system and other parts of the body (Gupta et al., 1971 & 1972). Inhibition of brain acetylcholinesterase (AChE) is generally regarded as a useful indicator of poisoning by OP or carbamate pesticides (Martin et al., 1981; Grue et al., 1983). Depression of AChE activity in brain is usually the most sensitive measure of toxicity. The inhibition of AChE in different species by a variety of OP pesticides is well established (Cohen et al., 1985; Elroaf et al., 1977). Evaluation of OP pesticides toxicity needs information on pharmacokinetic and enzyme kinetic studies. The information is very much essential on the time course of cholinesterase inhibition in in vivo experiments. Kinetic studies on species differences in AChE sensitivity to OP pesticides is presented by Wang & Murphy (1982). For some OP pesticides it may still be logical to base acceptable daily intake on data from adequate short term in vivo studies of anti-ChE activity since, such activity is the most sensitive criteria for effects by these compounds (WHO/FAO, 1972). In the present study the effect of short term exposure (single LD50 dose) of two OP pesticides monocrotophos (MCP) and abate on brain AChE is presented. A kinetic study on inhibition of rat brain AChE by MCP and abate produced a marked decrease in AChE activity. Both inhibition and the rate of reactivation of the inhibited enzyme in the presence of substrate have been determined at regular intervals of 1, 3, 5 and 7 days. The kinetic studies on in vitro inhibition of brain AChE by MCP is compared with in vivo studies.

## MATERIALS AND METHODS

All the reagents used in the present study were obtained from SIGMA and used without further purification. Technical grade of MCP (purity > 95%) was a gift from NOCIL, Bombay and abate (purity > 95.5%) from VOLHRO, Hyderabad.

Male Wistar strain rats weighing about 120 ± 5 gms used in the present experiment were obtained from Centre for Cellular & Molecular Biology, Hyderabad. They were housed in polypropylene cages (shoe box cage with stainless steel grill tops 29x22x13.5 cms.) maintained at 22 ± 2° C with 55±10% relative humidity at 12 hours dark and 12 hours light cycles. The animals had free access to food (supplied by Lipton India Ltd., Bangalore) and sterile drinking water. The rats were divided into two groups and orally administered LD50 doses of 20 mg/kg MCP and 8,600 mg/kg abate respectively.

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The rats in both the groups were sacrificed after single treatment, at time intervals of 1, 3, 5 & 7 days. The brains were isolated and homogenized (10% w/v in 0.1M phosphate buffer, pH 8.0) using Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate was centrifuged at 3000 x g for 10 min and the resultant supernatant was recentrifuged at 3000 x g for 10 min respectively and used as the enzyme source. All the enzyme preparations were made at 0 - 4° C. Protein was estimated by the method of Lowry et al., (1951). Assay of brain AChE was done as described by Ellman et al., (1961). The measurement of the rate of production of thiocholine as acetylthiocholine is hydrolysed was accomplished by the continous reaction of the thiol with 5:5-dithiobis-2-nitrobenzoate ion, to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The rate of colour production was measured at 412 nm in a Hitachi 100-10 Spectrophotometer by recording continuously for six minutes on a Perkin-Elmer - 56 recorder. The AChE activity was calculated per mg protein.

A typical run for all the experiments used 2.8 ml of 0.1M phosphate buffer pH 8, 50 µl (0.16mM) 5,5'-dithio-bis(2-nitrobenzoic acid), 50 µl (1mg) protein and 100 µl of substrate - acetylthiocholine iodide (0.020, 0.023, 0.025, 0.030, 0.035, 0.040, 0.050, 0.065, 0.1 & 0.2 mM).

In in vitro assay various concentrations of pesticide MCP were prepared in distilled water. 3 µl of each concentration of pesticide ( $1.87 \times 10^{-5}$ , $3.72 \times 10^{-5}$, $7.44 \times 10^{-5}$ and $11.2 \times 10^{-5}$ M ) along with various substrate concentrations were added simultaneously to react with the enzyme at 24 ± 1° C. Maximum velocity ($V_{max}$) and enzyme constant ($K_m$) values were computed from a detailed substrate concentration curves using Lineweaver-Burk (LB) transformations. The Inhibitor constant ($K_i$) was determined graphically from reciprocal plots made at different inhibitor concentrations. The slopes of intercepts of these lines were plotted against the inhibitor concentrations (Dixon & Webb 1965). The data represents mean of five animals each in triplicate.

## RESULTS AND DISCUSSION

The enzyme activity in the MCP treated animals has shown a maximum inhibition on day 1 with a significant recovery on day 3. It was again inhibited by day 5 (almost equivalent to day 1) and recovered by the day 7. Whereas, in abate the maximum inhibition was seen on day 3 with gradual recovery upto day 7. Earlier studies by Joseph (1987) report that rainbow trout brain AChE activity remained depressed for 8 days after 24 hrs exposure to 25 mg of Methamidophos/L and 15 days after 24 hr exposure to 400 mg Acephate. Six to fourteen days were necessary for brain ChE activity to recover to normal levels in three species of fish exposed to anticholinesterase cyclohexylmethyl phosphorofluoridate (Weiss, 1958). There appears to be a varied response to OP's among different species.

The equilibrium constant $K_m$ of the enzyme substrate complex of both MCP and abate treated animals at regular intervals of 1, 3, 5 & 7 days were graphically determined by applying LB plots as shown in Fig. 1. There was a decrease in $K_m$ in both MCP and abate when compared to control. Since, the assays are specific for AChE, the decrease may be partly due to the reduction in Kcat. However, we assume that there is no drop in AChE synthesis.

MCP has shown no significant change in the $K_m$ but, $V_{max}$ varied showing its noncompetitive nature. Whereas, abate showed change in both $K_m$ and $V_{max}$ at different time intervals. The change in $K_m$ may be due to the different forms of

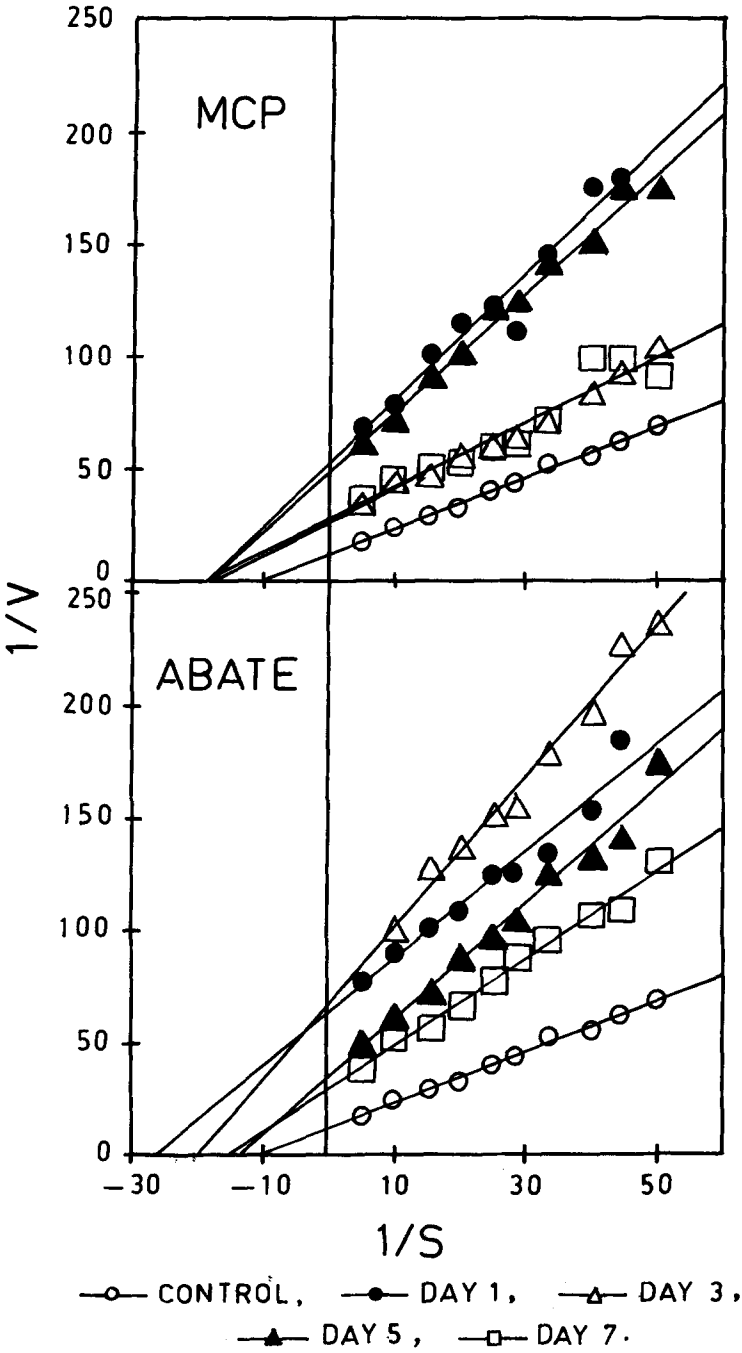


FIGURE 1. IN VIVO BRAIN AChE INHIBITION.

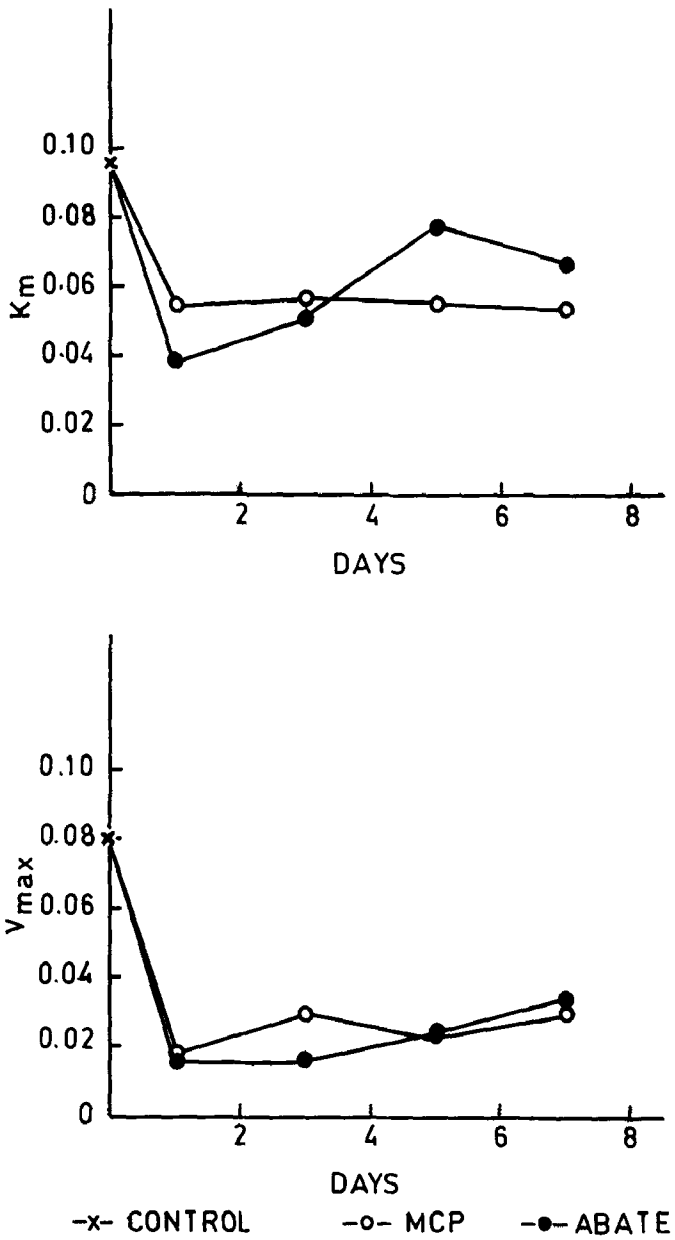


FIGURE 2. IN VIVO $K_m$ AND $V_{max}$ CHANGES WITH TIME

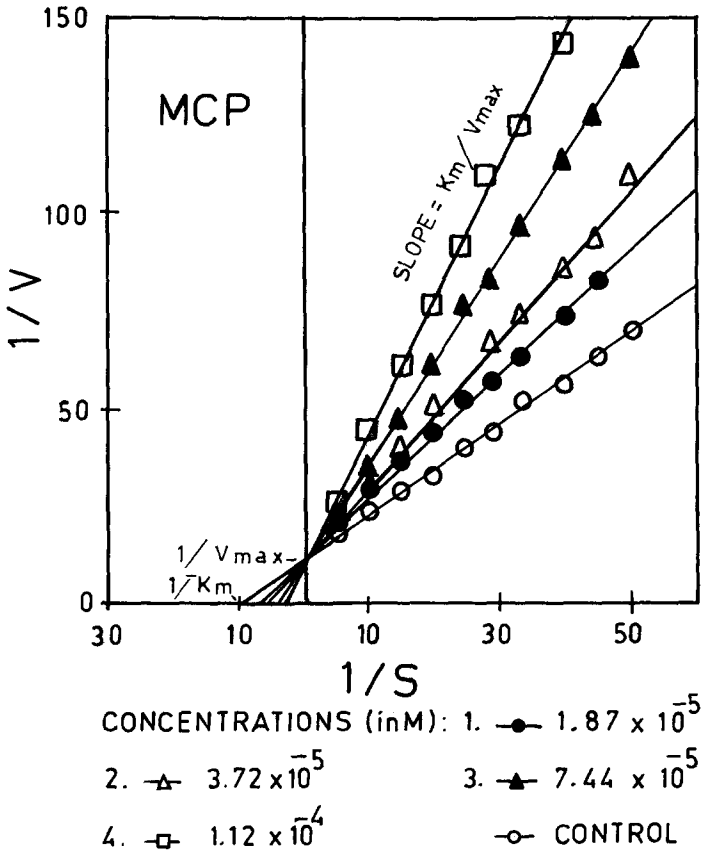


FIGURE 3. IN VITRO BRAIN AChE INHIBITION

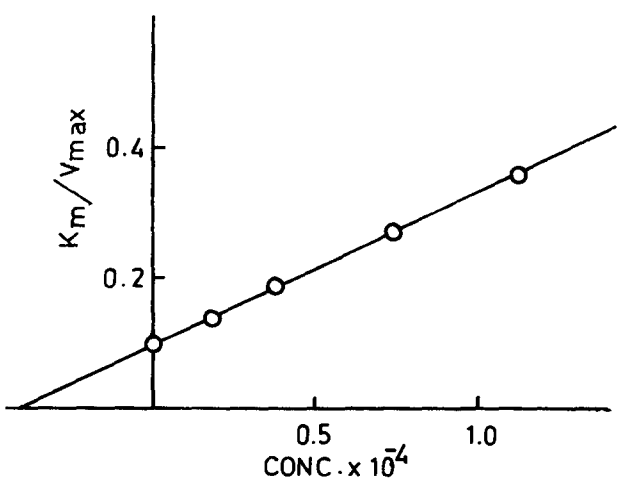


FIGURE 4. Ki VALUE OF MCP

the enzyme. The $K_m$ and $V_{max}$ values vs time are shown in Fig. 2. To be consistent with the in vivo MCP results, the in vitro studies conducted by preincubating the pesticide with enzyme showed no change in $K_m$ confirming its noncompetitive nature.

As seen in Fig. 3 the in vitro MCP experiments showed an increase in the $K_m$ with no change in the $V_{max}$, with increasing inhibitor concentration showing its competitive nature. Based on $K_m$ values at different concentrations of MCP, $K_i$ value was calculated and found to be $3.96 \times 10^{-5}$ M (Fig. 4). However, as shown by the in vivo studies the $K_m$ decreased and $V_{max}$ varied when compared to control. This may be due to phosphorylation and detoxifying mechanism controlled by various enzymes. The differences might also be due to nonspecific binding of OP's to proteins other than AChE (Benke & Murphy, 1974; Singh et al., 1985). The inhibitory powers of OP insecticides is governed by the affinity of insecticides for the enzyme active site and/or by the rate of phosphorylation (Main, 1964). The results have shown that the toxic properties based on in vitro studies may not be extrapolated to in vivo studies. Therefore, to assess the toxic nature of the pesticides in vivo studies have to be conducted.

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