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CHOLINESTERASE ACTIVITIES DURING ACUTE AND SUB-ACUTE PHOSPHAMIDON TREATMENTS IN RATS

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ABSTRACT: Organophosphate pesticides exert their toxic effects by cholinesterase inhibition and the consequent prolongation of the undesirable effects of accumulation of acetylcholine. However, as time elapses, sustained cholinesterase inhibition through sustained sub-acute administration of organophosphates leads to the gradual disappearance of initial signs of toxicity, termed behavioral tolerance. In the present study, cholinesterase activities in different regions of rat brain were examined upon the administration of acute and sub-acute doses of the organophosphate phosphamidon. Separate groups of male albino rats were given a one-time acute dose of 1/2 LD_{50} (6.64 mg/kg body wt/day), and a daily sub-acute oral dose of phosphamidon (4.333 mg, equivalent to 1/3 of LD_{50}) for 15 days. The activities of acetylcholinesterase (AChE), erythrocyte cholinesterase (EChE) and plasma cholinesterase (PChE) were estimated in both control and experimental groups. Activities of all the cholinesterases showed a decrease, and the decrease varied among different brain regions at different times. Maximal decrease under acute and sub-acute administrations was recorded from striatum. Greater inhibition of AChE and PChE activities was observed in 1 day after sub-acute administration, while EChE activity recorded highest decrease in 15 days of sub-acute administration. Recovery towards the controls under sub-acute dosing was observed with time for AChE and PChE but not for EChE. After 7 days, disappearance of majority of the signs and symptoms of toxicity was noticed as in our other studies, while the reduction in cholinesterase activities was found up to the 15^{th} day, indicating the development of behavioral tolerance to phosphamidon. Pseudocholinesterase activities seem to have a role in regulating homeostasis of cholinesterases and in the development of symptomatic tolerance to phosphamidon.

Key words/phrases: Cholinesterases, Erythrocytes, Phosphamidon, Plasma Rat brain, Tolerance.

INTRODUCTION

Most poisons, including insecticides, owe their toxicity to their effect on the nervous system. Majority of organophosphorus compounds (OPs) share the common feature of being potential inhibitors of cholinesterases (Matsumura, 1985; Kwong, 2002**).** Organophosphorus compounds (OPs) inhibit

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acetylcholinesterase (AChE) activity, resulting in the accumulation of acetylcholine (ACh) at cholinergic synapses leading to paralysis and finally death (O'Brien, 1960; Singh and Sharma, 2000; Balali Mood *et al*., 2006). The cholinergic excess resulting on OP intoxication leading to hyper-stimulation of the postsynaptic receptors is thought to be responsible for the acute lethality of OP compounds (Valdes *et al.,* 1988). Neurochemical studies on organophosphate toxicity in animals have emphasized that majority of the findings are related to the deficits of cholinergic system (Arnal *et al*., 1990). The depression of erythrocyte cholinesterase (EChE) and plasma cholinesterase (PChE) activity levels has been used as a sensitive indicator of exposure to OPs (Trundle and Marcial, 1988).

Sub-chronic and chronic exposure to low doses of majority of OP compounds leads to the development of tolerance, wherein the animals look apparently normal despite cholinergic impairment (Russell and Overstreet, 1987; Swamy *et al.,* 1993).

Brain AChE activity and ACh levels were found to be variable in different areas in rat brain. Similarly, cholinesterase activities in plasma and erythrocytes were found to differ. Pseudocholinesterases show greater affinity to butyrylcholine than to acetylcholine, and have greater sensitivity to organophosphate inhibitors (La Du and Lockridge, 1986). BuChE and aliesterase are known to decrease the AChE inhibition wherein these two enzymes bind to the toxic compound thereby reducing the AChE inhibition (Desai and Joshi, 1985; Pla and Johnson, 1989). Pseudocholinesterases for sometime have been implicated as the effective reducers of inhibitory power of many OPs (Pla and Johnson, 1989).

The investigation of Rahman and Siddiqui (2003) with phosphorothionate (RPR-V) revealed significant inhibition of erythrocyte cholinesterase which is a target enzyme for organophosphorus compounds, and further revealed its effect on normal synaptic transmission. OP pesticides such as monocrotophos (MCP), dichlorvos (DDVP) and phosphamidon significantly inhibit both MAO-A and MAO-B activities in rat brain mitochondria (Nag and Nandy, 2001). The most widely used diagnostic tests for OP exposure are the estimations of plasma cholinesterase (PChE) and erythrocyte cholinesterase (EChE) activities (Karalliedde, 1999). However, no correlation has been observed between the clinical state and PChE/ EChE activity levels, as the acute effects of OP compounds depend upon the inhibition of AChE in brain and neuromuscular junctions (Yilmazalar and Ozyurt, 1997).

The organophosphorus compound phosphamidon is primarily used as an insecticide and secondarily as an acaricide, with toxicity matching those of the other organophosphates such as monocrotophos, phosalone etc. It is a cholinesterase inhibitor with rapid contact and stomach action. The technical product is very highly toxic to mammals and is listed in WHO Hazard Class Ia. Available reports indicate that the relative sensitivities of various cholinesterases have not been adequately established. The present study examines the relative sensitivities of cholinesterase activities of erythrocytes, plasma and different regions of rat brain and also elucidates the comparative inhibition of cholinesterase activities during acute and sub-acute phosphamidon treatment.

MATERIALS AND METHODS

Male Wistar rats (150 ± 2 g) were selected as experimental animals in the present study. They were maintained in the animal house at 25 ± 2 °C with a photoperiod of 12:12 h light and dark and 70% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum.*

Technical grade phosphamidon (2-chloro-3-(diethylamino)-1-methyl-3-oxo-1-propenyl dimethyl phosphate) was obtained from CIBA-GEIGY, Mumbai, India, in liquid form which is highly soluble in water. Hence water is selected as a vehicle for administration by oral intubation. Six batches of rats with six in each batch were taken and median lethal dose (LD_{50}; 13.29 mg/kg body wt/day) was determined by Probit method of Finney (1971). An oral dose of 1/3rd LD_{50} (4.433 mg/kg body wt/day) was selected for daily dosing for 15 days as sub-acute dose. Similarly 1/2 LD_{50} (6.64 mg/kg body wt/day) was selected as acute dose. Different areas of the brain such as cerebral cortex (CC), cerebellum (CB), striatum (ST), hippocampus (HC) and pons-medulla (PM) were isolated using standard anatomical marks (Glowinski and Iverson, 1966). Blood was collected from rats by using a heparinized syringe. The supernatant was collected to estimate plasma cholinesterase. The hemosylate was used for the estimation of erythrocyte cholinesterase.

Estimation of Cholinesterases

Acetylcholinesterase (AChE), erythrocyte cholinesterase (EChE) and plasma cholinesterase (PChE) were estimated by the method of Ellman *et al*. (1961) with slight modifications.

RESULTS

Brain cholinesterase (BAChE), erythrocyte (EChE) and plasma (PChE) cholinesterase activities were estimated during acute and sub-acute phosphamidon treatments in rats. During acute phosphamidon treatment the activities of all these cholinesterases were inhibited. Under acute dosing the inhibition of BAChE was highest in striatum (ST) followed by PM, HC, CC and CB. EChE activity showed marked inhibition but PChE activity was less affected (Table 1).

Table 1. Changes in cholinesterase activity levels in different brain regions, erythrocytes and plasma during acute and sub-acute phosphamidon treatment.

Region	Control	Acute Dose	Sub-acute Dose		
			1 Day	7 Days	15 Days
Cerebral Cortex	3.47	1.22	1.77	1.91	1.99
± SD	0.49	0.38	0.18	0.82	0.73
		(-65.00)	(-49.01)	(-44.96)	(-42.65)
Cerebellum	3.01	1.85	2.28	2.45	2.73
± SD	0.39	0.37	0.45	0.76	0.65
		(-38.54)	(-24.25)	(-18.61)	(-9.31)
Striatum	20.35	5.39	10.67	12.13	12.01
± SD	2.81	0.47	2.80	2.91	2.27
		(-73.51)	(-47.57)	(-40.39)	(-40.98)
Hippocampus					
± SD	3.17	1.08	1.69	2.08	2.29
	0.94	0.36	0.28	0.47	0.63
		(-65.93)	(-46.69)	(-34.39)	(-27.76)
Pons-Medulla					
± SD	4.55	1.51	2.08	2.45	3.04
	0.88	0.42	0.49	0.20	0.77
		(-67.04)	(-54.26)	(-46.15)	(-33.33)
Erythrocytes					
± SD	2.71	0.95	1.59	0.88	0.51
	0.54	0.21	0.75	0.08	0.21
		(-64.95)	(-41.33)	(-67.53)	(-81.18)
Plasma					
± SD	0.67	0.26	0.31	0.34	0.44
	0.13	0.08	0.05	0.08	0.07
		(-61.20)	(-53.73)	- 49.45	(-33.43)

All values are significant at $P < 0.001$
All the values are mean ± standard deviation (SD) of six individual observations.
Values in parentheses are percent changes from the control.

The cholinesterase activity in different brain regions was also found significantly inhibited one day after administration of sub-acute dose of phosphamidon. Erythrocyte cholinesterase activity was inhibited significantly under both acute and sub-acute treatments. Recovery in BAChE activity towards the controls was observed on subsequent

treatments whereas the inhibition of EChE was found to increase with the duration of treatment. In the present experimental schedule, maximum decrease in EChE activity was observed after 15 days of sub-acute phosphamidon treatment (Table 1).

PChE activity was significantly inhibited after 7 days of sub-acute treatment. However, the inhibition was less when compared with that after one-day treatment. The inhibition decreased further after 15 days of treatment when compared with 1 and 7 days of phosphamidon treatment (Table 1).

Cholinesterase inhibition was calculated after 1, 7 and 15 days of sub-acute phosphamidon treatment. The recovery of AChE was higher in cerebellum, followed by HC, PM, ST and CC. It could be clearly noted from the trend that different brain areas were significantly affected by phosphamidon treatment. EChE activity showed greater inhibition without recovery during different periods of phosphamidon treatment (Table 1).

DISCUSSION

Organophosphates are known to inhibit cholinesterases through phosphorylation of the enzyme (Gary and Duggleby, 1989), leading to acetylation and prevention of acetylcholine from combining with the active site. The extent of damage in terms of ChE inhibition by phosphamidon in different brain areas and inhibition of other cholinesterases is poorly understood. The present results show varied inhibitory patterns of cholinesterase activities upon phosphamidon toxicity.

Among different regions of the brain, the highest cholinesterase activity was recorded from striatum, and the lowest from cerebellum in the present study. The reports of other investigators (Stavinoha *et al.,* 1976; Ehlert *et al.,* 1980) are in agreement with this finding. In the present study, the cholinesterase activities were inhibited in different regions of the brain following acute treatment of rats with phosphamidon. From the results it is clear that the sensitivity of AChE varies with the region of the brain, which could be attributed to the differential cholinergic innervations in different regions. The overt behavioral symptoms by anti-ChE-OPs could be due to cumulative effects in all the brain regions of the rat. In the present study the rats treated with sub-acute phosphamidon daily for 15 days were able to adapt to reduced levels of cholinesterase activities. It is also clear that marked variation exists in the degree of inhibition among different regions of the brain and also during different time periods of the treatment schedule. It was reported earlier that the striatal AChE activity was inhibited more in

malathion-tolerant animals (Bartholomew *et al*., 1985), and hippocampal and striatal AChE was more inhibited in disulfoton-tolerant rats (Costa and Murphy, 1983).

Thus, the adaptation of the nervous system to cholinesterase inhibitors depends on the duration and the level of exposure and the type of inhibitor. It is also interesting to observe a marked recovery from AChE inhibition during different days of sub-acute phosphamidon treatment, which presumably could be due to stepped-up synthesis of the enzyme. The overt behavioral symptoms of toxicity were such as tremors, convulsions, lachrymation, urination, defecation etc. Normalcy in behavior could be restored earlier than the recovery in enzyme activity or before ACh levels return to normal (Hoskins and Ho, 1992).

Similar type of recovery was reported during low doses of monocrotophos (Desai and Joshi, 1985; Swamy *et al*., 1992). From the results, it is obvious that the AChE activity showed greater inhibition after 1-day treatment but recovered during subsequent dosing regimen as evidenced by reduced AChE inhibition in brain and plasma after 7 days and 15 days of phosphamidon treatment. Earlier recovery of AChE in CB and HP is interesting as these areas are associated with motor functions, learning and memory.

It was also reported that OP compounds may exert effects on neuronal function by phosphorylating the enzymes and proteins other than AChE. Treatment with the toxicant phosphorothionate (RPR-V) revealed that the aspartate (AAT) and alanine (AlAT) aminotransferase activities increased in serum, but they decreased significantly in liver and lung, indicating necrosis of these tissues. However, in the case of kidney the level of these enzymes increased significantly parallel to that in serum, which is suggestive of an increased synthesis of these enzymes as a possible adaptive mechanism to toxicant-stress. These biomarker enzymes can be detected rapidly and hence may be used for the prediction and diagnosis of pesticide insults (Rahman and Siddiqui, 2003).

Phosphamidon significantly inhibits both monoamine oxidase-A and -B (MAO-A and MAO-B) activities in rat brain mitochondria, suggesting that the mechanism of action of OP pesticides is through phosphorylation of serine residue present in the active center of MAO (Nag and Nandy, 2001). Organophosphate insecticide chlorpyrifos and the carbamate insecticide carbaryl showed their effects with MAO-A activity unaffected. Acute carbaryl administration produced AChE and BuChE inhibition and also

caused a significant decrease in 5-HT uptake but no change in MAO-A activity. Interference with the 5-HT system by chlorpyrifos and carbaryl could contribute to the toxicity (Magda *et al*., 2001).

The erythrocyte AChE (EChE) activity was significantly inhibited during acute phosphamidon treatment. EChE activity was inhibited in rats treated dermally with varied concentrations of phosphamidon. The plasma (pseudo) cholinesterase (PChE) was also inhibited during acute phosphamidon treatment, and the extent of inhibition was less when compared to that of brain AChE. Plasma pseudocholinesterase activity was inhibited during sub-acute phosphamidon treatment, which decreased gradually during the development of behavioral tolerance. The development of behavioral tolerance, even after cholinesterase inhibition, was demonstrated, as behavioral tolerance develops when animals are exposed to OP compounds for longer durations (Potter *et al.,* 1984). Reports on cholinesterases during behavioral tolerance indicated both continued inhibition of the enzyme as well as partial recovery of the enzyme at later periods (Sivam *et al*., 1984; Russell *et al*., 1986) with or without recovery in AChE activity (Sivam *et al*., 1983; Gupta *et al*., 1985).

With regard to the sensitivities of cholinesterases, it was observed that brain AChE activity, particularly in striatum, was inhibited more than EChE and PChE activities during acute phosphamidon treatment. Similarly, sub-acute phosphamidon treatment exerted greater inhibition on brain AChE (pons-medulla after 1-day and 7-days treatment) as compared to EChE and PChE. These observations in general suggest that brain AChE is a more sensitive indicator than the other two cholinesterases. These observations are in concurrence with the reports of Boiko (1977) who also reported that the brain AChE is a more sensitive indicator of dietary diazinon than plasma and erythrocyte cholinesterases.

However, EChE activity was inhibited to a greater extent than the PChE and BAChE activities after 15 days of sub-acute treatment. Though the reasons for the variation in activities of different cholinesterases are not understood from the present study, it is presumably an adaptive mechanism to balance the changes in cholinesterase activities in the other segments. The role of pseudocholinesterases during the toxicity of and tolerance to OP compounds is not clear. Their presence is significant in plasma and blood vessels of brain (Friede and Fleming, 1964). Popularly known as scavengers or sinks, these enzymes found in plasma and erythrocytes have the ability to reduce the concentration of toxic chemicals (OP) entering the CNS (Clement, 1984;

Sterri and Fonnum, 1984).

It is evident that cholinesterases from different compartments apparently exhibit a characteristic time-dependent variation in the sensitivities, which may be in part responsible for the phenomenon of behavioral tolerance induced by OP compounds. Role of PChE by its recovery from inhibition also seems to contribute to this. The varied effects of phosphamidon in different brain areas showed further that the potential toxicity of OP compounds differs from chemical to chemical and among different brain regions (Singh, 1985). Homeostatic mechanisms by way of pseudocholinesterases taking over the role of true cholinesterases may as well be in operation (Murali Mohan *et al*., 1988a, b).

The present study reveals inhibition of activities of brain cholinesterase as well as erythrocyte and plasma cholinesterases in rats under both acute and sub-acute intoxication with phosphamidon. The degree of inhibition differs between different areas of the brain. The overt behavioral symptoms of cholinesterase inhibition return to normalcy even before the cholinesterase activity levels return to normal levels, indicating the operation of other homeostatic mechanisms.

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