African Research Review

An International Multi-Disciplinary Journal, Ethiopia
Vol. 3 (2), January, 2009
ISSN 1994-9057 (Print) ISSN 2070-0083 (Online)

Phosalone-Induced Changes in Regional Cholinesterase Activities in Rat Brain during Behavioral Tolerance *(pp 20-30)*

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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. The signs of toxicity include tremors, convulsions, lachrymation, defecation etc. However, sustained cholinesterase inhibition through sustained administration of organophosphates would lead to the gradual disappearance of the initial signs of toxicity over time, termed behavioral tolerance. The present study was undertaken to examine the activity levels of cholinesterases in different regions of rat brain during the development of behavioral tolerance to the organophosphate phosalone. Male albino rats were given a daily oral dose of phosalone (41.35 mg, equivalent to ¼ of LD_{50}) every day for 15 days and the activities of acetylcholinesterase (AChE) butyrylcholinesterase (BuChE) and

pseudocholinesterase (PChE) were estimated at intervals at 1, 3, 9 and 15 days of treatment. All the cholinesterases were inhibited, and this inhibition was found to vary among different brain regions at different times. Greater inhibition of AChE and BuChE activities was observed at 9 days, while for PChE it was recorded at 3 days. Recovery trend to normalcy was observed earlier in PChE compared to AChE and BuChE. The signs and symptoms of pesticide toxicity were mainly cholinergic. Inhibition of cholinesterases was well correlated with the appearance and severity of signs and symptoms. Tremors and convulsions in particular were more after 9 days. After 9 days, decline followed by disappearance of majority of the signs and symptoms was noticed while reduction in cholinesterase activities still continued, indicating the development of behavioral tolerance to phosalone. Among the brain regions, striatum recorded a greater decrease in cholinesterase activity. Earlier recovery of pseudocholinesterase activity seems to be an interesting phenomenon in regulating homeostasis of cholinesterases and in the development of symptomatic tolerance of phosalone.

Key words: Phosalone, Cholinesterases, Rat brain, Behavioral tolerance.

Introduction

Organophosphate (OP) compounds act on the cholinergic system by inhibiting cholinesterases, leading to a marked increase of acetylcholine level in central and peripheral synapses (Arnal *et al*., 1990). Phosalone is a non-systemic dithiophosphate insecticide and is used as a substitute for DDT in agricultural and domestic fronts. It has been reported that OP compounds differ greatly in their potency of inhibition (Singh, 1985). Sub-chronic and chronic exposure to low doses of majority of OP compounds leads to the development of tolerance (Russell and Overstreet, 1987; Swamy *et al.,* 1993). Biochemical and behavioral alterations were noticed along with receptor mechanisms during tolerance development (Russell *et al.,* 1986; Van Dongen and Wolthuis, 1989). Cholinergic system is mainly involved in this process of tolerance. Pseudocholinesterases for sometime have been implicated as the effective reducers of inhibitory power of many OPs (Pla and Johnson, 1989).
In the present investigation the effect of phosalone on cholinesterases in different brain regions and the possibility of phosalone-induced tolerance (symptomatic) were assessed during the treatment period of 15 days.

Methodology

Male albino Wistar rats weighing 130±20 g were used. Four animals were housed per cage and allowed access to food and water *ad libitum.* Technical grade (98% purity) phosalone [O, O- diethyl-s- (6-chloro-1-3 ben 30XO3ol-2(3H)-O-methyl) phosphorodithiote], obtained from Volhro Ltd., India was used. Control animals received normal saline and the experimental rats received it along with phosalone. LD_{50} values were determined by Probit method (Finney, 1971). ¼ LD_{50} of phosalone was given by oral intubation daily for 15 days. Signs and symptoms were noted at regular intervals of 3h, 6h, 12h, and 24h and pooled later. The observations of tremors and convulsions were quantified into arbitrary units in the scale of 0=no signs, 0.2=slight (slow tremor on head); 0.4=moderate (faster tremor of the head trunk and limbs); 0.6=high (more intense tremors) and 0.8=severe. Similar scale was developed for convulsions, viz., 0.0=none, 0.1=slight, 0.2 moderate and 0.3 high.

Both control and experimental animals were sacrificed by cervical dislocation and the brain areas, viz. the cerebral cortex (CT), cerebellum (CB), hippocampus (HP), striatum (ST) and medulla (MD) were quickly dissected out from the isolated brains following the standard anatomical markings (Glowinski and Iverson, 1966) on to a chilled Petri dish placed on crushed ice. The activities of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and pseudocholinesterase (PChE) were estimated by the method of Ellman *et al*. (1961). For estimating BuChE and PChE activities, true cholinesterase inhibitor BW 284 C51 dibromide was used (20mM). The enzyme activities were expressed as µmoles of acetylthiocholine/ butyrylthiocholine hydrolyzed/mg protein/min.

Mann Whitney 'U' test for behavioral scores, and one way ANOVA by following the Student's Newman-Keuls test were used to analyze the statistical significance. P values of 0.05 or less were considered significant.

Results

The signs and symptoms observed were tremors, convulsions, involuntary urination, defecation, lachrymation, salivation and sweating. These signs and symptoms were developed after 12h to 24h of oral dosing. The tremors commenced from 2nd day, reaching the peak in terms of severity on the 9th day from where tremors showed a downward trend with a steep fall from the 12th day, returning to normalcy by the 15th day. Convulsions were found to

set in only from the 5th day. The convulsions were always slight to moderate in the scale with peak on the 9th day (data on other signs and symptoms are not shown). The distribution of cholinesterases in the brain regions was found in the order of AChE: ST>HP>CT>MD>CB; BuChE: ST>MD>HP>CT>CB; PChE: MD>HP>ST>CT>CB. After 24 h the inhibition of BuChE was found to be greater than that of AChE and PChE. After 3 days the PChE activity was inhibited more than AChE and BuChE activities. Both AChE and BuChE activities were found inhibited when recovery in PChE activity was noticed after 9 days.

Further recovery in AChE and BuChE activities was noticed by 15 days, which was in general comparatively less than that in PChE. Among the brain regions the AChE activity of cortex was inhibited more after 1 day (69%) whereas in almost all the areas BuChE activity was inhibited (60-85%) more in striatum followed by hippocampus and cortex. PChE activity was inhibited less in cortex when the inhibition of (35-45%) was noticed in other areas. Interestingly, greater inhibition of PChE was noticed in striatum followed by medulla, hippocampus and cerebellum. AChE inhibition was progressive in all the areas from the 1st day to the 3rd and 9th day (except in cerebellum). BuChE inhibition was noticed after 3 days compared to the recovery trend in 1 day and the inhibition of PChE was further progressive after 3 days in all the brain areas. Maximum PChE inhibition was observed in striatum. After 9 days, recovery was noticed in PChE activity in all areas (inhibition ranged from 5 to 75%). Recovery from BuChE inhibition was noticed only in medulla and cortex. In other areas the inhibition was progressive (39-83%). After 15 days, recovery in AChE, BuChE and PChE activities compared to 9 days was noticed. Greater AChE recovery was observed in cerebellum whereas greater BuChE recovery was seen in cerebellum and medulla. Greater PChE recovery was seen in striatum and cerebellum. Hippocampal PChE remained inhibited by 50% even after 15 days (Table 1). In tremors and convulsions the severity was more after 9 days when maximum AChE and BuChE inhibition was noted in several brain areas.

Discussion

Cholinergic impairment during OP toxicity is a well understood phenomenon. However, the extent of damage in terms of ChE inhibition by phosalone on different brain areas and on other cholinesterases is poorly

understood. The present results show varied inhibitory patterns of cholinesterases upon phosalone toxicity. Earlier studies carried on frog brain areas showed Inhibition of AChE by phosalone (Balasundaram and Selvarajan, 1990). Their observations are in agreement with the present results in rat brain areas and also demonstrate the development of symptomatic tolerance to phosalone by the animals, as judged by the gradual disappearance of overt manifestations of toxicity like tremors and convulsions with multiple sub-chronic dosing over an extended period of time (Russell *et al*., 1986; Swamy *et al*., 1993). Frequency and magnitude of signs and symptoms like tremors and convulsions are dependent on the inhibition of AChE activity. Striatum is the area associated with tremors and convulsions (Siegel, 1991). In the present study we have noticed the inhibition of AChE and BuChE activities by 89% and 82% respectively in striatum when peak levels of tremors and convulsions were observed (Table 1). Normalcy in behavior was restored earlier than the recovery in enzyme activity or before ACh levels return to normal (Hoskins *et al*., 1986). It could be this possibility in the present study accounting for the disappearance and decline of tremors and convulsions from the 9^{th} day to the 15^{th} day, in addition to the recovery of both AChE and BuChE activities to a major extent. Recent studies emphasize that subsensitivity of muscarinic receptors leads to the development of tolerance and this subsensitivity was noted when 55% AChE inhibition occurred (Russell *et al*., 1975). According to this notion, during symptomatic behavioral tolerance to phosalone receptor mechanisms appear to be in operation. Van Dongen and Wolthius (1989) further demonstrated that more than a simple alteration of the number of receptors, a non-competitive modulation of muscarinic receptor binding is involved in the development of behavioral tolerance. This area is to be looked into for further understanding the mechanism of phosalone-induced tolerance.

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 and Gupta *et al*., 1985). In the phosalone-induced tolerance, recovery of AChE activity seems to be in operation. The inhibition of cholinesterases was found be varied among brain regions. Striatal ACE activity was inhibited more in malathion-tolerant animals (Barthalomew *et al*., 1985), hippocampal and striatal AChE was

more inhibited in disulfoton-tolerant rats (Costa and Murphy, 1982). In the present study, greater inhibition of AChE and BuChE was observed after 9 days in striatum with lower inhibition initially. More number of cholinergic neurons in striatum (Ehlert *et al*., 1980) and greater activity in this region might have reduced the effect of phosalone initially which, however, did not continue as multiple doses of phosalone were given. Earlier recovery of AChE in CB and HP is interesting as these areas are associated with motor functions, learning and memory.

The role of pseudocholinesterases during the toxicity/tolerance of 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).

BuChE was found to decrease the inhibitory power of many OPs (Pla and Johnson, 1989). Role of BuChE during the development of tolerance to monocrotophos was earlier suggested (Swamy and Murali Mohan, 1992). In the present study earlier response of BuChE, that is greater inhibition after 1 day then AChE, gains significance as it shows reacting first to phosalone toxicity than AChE in brain areas. Greater inhibition of PChE after 3 days is also interesting. It appears that these enzymes have their role in the development of tolerance, particularly earlier recovery of PChE in striatum and cerebellum by 15 days.

Conclusions

The present study demonstrates the occurrence of behavioral/symptomatic tolerance to ¼ LD50 daily dose of phosalone as the dosing is continued. BuChE and PChE activities by their recoveries from inhibition seem to contribute to this. The varied effects of phosalone on different brain areas shows 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).

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Table 1: Changes in AChE , BuChE and PChE activities in different brain regions of male albino rats, exposed to a 1/4LD50 of phosalone for 15 days

		AChE (µmoles of acetylthiocholine iodide hydrolyzed/mg protein/min)								
S/No	Region	Control	1 Day	%change	3 Days	%change	9 Days	%change	15 Days	%change
1	CT	5.08±0.63	1.58±0.13	-68.90	1.56±0.07	-69.29	1.27±0.6	-75.00	3.42±0.6	-32.68
2	CB	3.18±0.94	1.94±0.16	-38.99	1.43±0.08	-55.03	1.63±0.19	-48.74	3.43±.31	+7.86
3	ST	21.20±0.72	16.14±0.37	-23.87	13.46±0.56	-36.51	2.22±0.98	-89.53	12.35±2.7	-41.75
4	HP	6.97±0.29	5.65±0.37	-18.94	2.61±0.19	-62.55	2.54±0.52	-63.56	5.6±0.32	-19.66
5	MD	4.55±0.88	3.17±0.13	-30.33	2.9±0.31	-36.26	2.95±0.08	-35.17	3.12±0.38	-31.43
		BuChE (µmoles of butyrylthiocholine iodide hydrolyzed/mg protein/min)								
1	CT	0.51±0.08	0.1±0.005	-80.32	0.21±.03	-58.82	0.23±.06	-54.90	0.25±0.03	-50.98
2	CB	0.38±0.03	0.15±.006	-60.50	0.23±.05	-39.47	0.33±0.02	-13.16	0.36±0.03	-5.26*
3	ST	0.72±0.01	0.11±.002	-84.70	0.07±.02	-90.28	0.30±0.02	-58.33	0.48±.02	-33.33
4	HP	0.58±0.19	0.11±.004	-81.03	0.10±.007	-82.76	0.28±0.04	-51.73	0.34±0.01	-41.38
5	MD	0.62±0.07	0.17±.002	-72.60	0.26±.04	-58.07	0.45±0.05	-27.42	0.61±0.03	-1.61*

		PChE (µmoles of acetylthiocholine iodide hydrolyzed/mg protein/min)								
1	CT	0.23±0.06	0.17±0.03	-26.09	0.13±0.003	-43.48	0.17±0.003	-26.09	0.21±0.07	-8.70*
2	CB	0.20±0.02	0.13±0.005	-35.00	0.03±0.002	-85.00	0.19±0.08	-5.00*	0.22±0.04	+10.00
3	ST	0.28±0.07	0.13±0.002	-53.57	0.02±0.005	-90.00	0.24±0.05	-14.29	0.28±0.05	0.00
4	HP	0.32±0.01	0.08±0.01	-75.00	0.05±0.02	-84.38	0.16±0.004	-50.00	0.20±0.04	-37.50
5	MD	0.56±0.22	0.26±0.02	-53.57	0.07±0.03	-87.50	0.31±0.05	-44.64	0.44±0.08	-21.43

Values are ± SD of six individual observations of six animals each. Control values are ±SD of 8-10 individual observations of 6 animals each. Values are significantly different from controls at $P < 0.05$ (SNK test). *Indicates no significance.