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A COMPARATIVE STUDY OF DELAYED NEUROTOXICITY IN HENS FOLLOWING REPEATED ADMINISTRATION OF ORGANOPHOSPHORUS COMPOUNDS

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Abstract: Hens treated with Mipafox (10 mg/kg, sc), sarin (50 μg/kg, sc) or parathion (1 mg/kg, sc) daily for 10 days exhibited severe, moderate and no ataxia respectively on 14th day after the start of exposure. The neurotoxic esterase (NTE) activity was significantly inhibited in the brain, spinal cord and platelets of hens treated with mipafox or sarin whereas no change was noticed with parathion treatment. All three compounds significantly inhibited acetylcholinesterase (AChE) activity in the platelets. Spinal cord of hens treated with mipafox, sarin or parathion showed axonal degeneration heavy, moderate and none respectively. It is concluded that repeated administration of equitoxic doses of mipafox, sarin and parathion to hens are marked, moderate and non-delayed neurotoxic respectively.

Key words: delayed neurotoxicity organophosphorus compounds NTE axonal degeneration ataxia

INTRODUCTION

Organophosphorus (OP) compounds are widely used in agriculture, industry and also as potent chemical warfare agents (1). Some of these compounds, beside producing acute cholinergic toxicity, have been reported to induce delayed neurotoxicity in human, hen and certain species of mammals (2, 3). OP compound-induced delayed neurotoxicity (OPIDN) is a syndrome which is characterised by a delay period of 4-21 days after intoxication before the clinical symptoms (ataxia and paralysis) are manifested (3). The initial biochemical step is inhibition of the enzyme neurotoxic esterase (NTE) of the nervous system (4). Minimum 70% NTE inhibition after single exposure and 45% after multiple exposure to OP compounds and subsequent aging of NTE is biochemical prerequisite for the development of OPIDN (5, 7). Morphologically there is axonal degeneration followed by demyelination (2). Recently a peripheral biochemical marker for OPIDN has been demonstrated (5). Adult hens are used to provide a model of OPIDN for laboratory studies (2). Since most of the previous studies have been performed in hens after a single exposure of OP compounds, however, population in general, military troops and farm animals may also be exposed to these compounds repeatedly. The aim of the present study was to assess the delayed neurotoxic effects in hens using behavioural, biochemical and histopathological analysis following repeated administration of equitoxic doses of three OP compounds - mipafox, sarin and parathion.

METHODS

Twentyfour adult white leghorn hens (Gallus domesticus) weighing 1.01-1.35 kg were used. They were divided into 4 groups of 6 hens each and housed

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in aluminium cages (2 per cage) and were provided food and tap water *ad libitum*. Hens in group 1 were administered normal saline subcutaneously daily for 10 days and served as control. Those in group 2, 3 and 4 were injected with mipafox (10 mg/kg, sc), sarin (50 µg/kg, sc) and parathion (1·0 mg/kg, sc) respectively daily for 10 days. The doses of three compounds administered were 1/10th of their respective LD_{50} values. The birds were carefully observed for occurrance of delayed neurotoxic symptoms (ataxia and paralysis) and assessed on 4-point scale as described by Sprague et al (6). Birds were sacrificed on 14th day after the start of the exposure.

Platelets were isolated from the blood as described previously (5). Neurotoxic esterase (NTE) was assayed in the brain, spinal cord and platelets according to the method of Johnson (7). Acetylcholinesterase (AChE) was assayed in the platelets by the method of Ellman et al (8).

Specimens of the spinal cord were fixed in neutral phosphate buffered formalin and embedded in paraffin. Paraffin sections (10 um) were stained with hematoxylin and eosin with luxol fast blue and Holm's silver stain and gold chloride (9) for examination under light microscope. The data were analysed statistically by Student's 't' test.

RESULTS

Mipafox treated hens exhibited severe ataxia whereas hens treated with sarin developed moderate ataxia on 14th day after the start of the exposure. Those treated with parathion did not show any delayed neurotoxic symptoms (Table I).

A significant inhibition of NTE activity in the brain, spinal cord and platelets was noticed in hens treated with mipafox and sarin. However, no significant change in enzyme activity was observed in hens treated with parathion (Table I). Acetylcholinesterase (AChE) activity in the platelets was significantly inhibited in hens treated with mipafox, sarin or parathion (Table I). Spinal cord section of mipafox treated birds showed heavy degeneration of axon and myelin of the lateral columns of cord (Fig. 1). Reticular fibers of small blood vessels were also frequently impregnated. Degeneration caused by sarin was moderate (Fig. 2) and axonal swellings were accompanied by macrophages accumulation.

TABLE I : Effect or repeated administration of mipafox, sarin and parathion on behavioural pattern (ataxia) and NTE in various tissues and AChE in the platelets of hens after 14 day exposure. Each value represents mean±S.E.

Groups	*Ataxia*	*Neurotoxic esterase (NTE)*[a]			*Acetylcholine-*[b] *sterase (AChE) platelets*
		Brain	*Spinal cord*	*Platelets*	
Control (n = 6)	-	2177·0 ±26·5	791·0 ±12·6	10·80 ±0·64	1091·5 ±20·4
Mipafox (n = 6)	++	689·5* ±26·4	401·6* ±10·3	4·74* ±0·57	409·9* ±8·5
Sarin (n = 5)	+	1013·6* ±26·1	488·2* ±13·1	4·93* ±0·30	310·6* ±9·2
Parathion (n = 6)	—	2087·9 ±42·2	750·0 ±16·9	10·85 ±0·78	501·7* ±15·9

Atxia : + Moderate ++ Severe
a = n moles of phenyl valerate hydrolysed /min/gm tissue or mg of platelet protein.
b = n moles of acetylthiocholine hydrolysed/min/mg protein.
* = v/s control ($P < 0·001$).

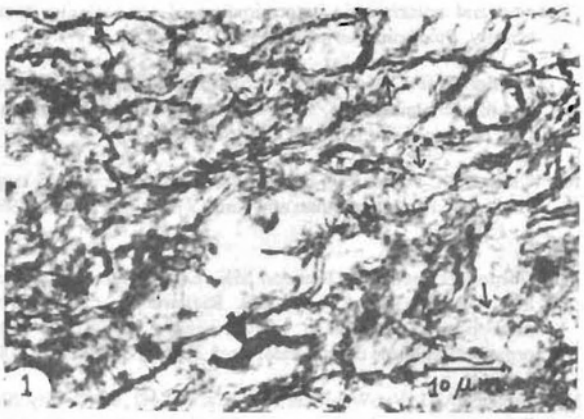

Fig. 1 : Mipafox exposed illustrating axonal degeneration. Arrows indicate axonal degeneration while arrow head indicates capillary reticular fibers.

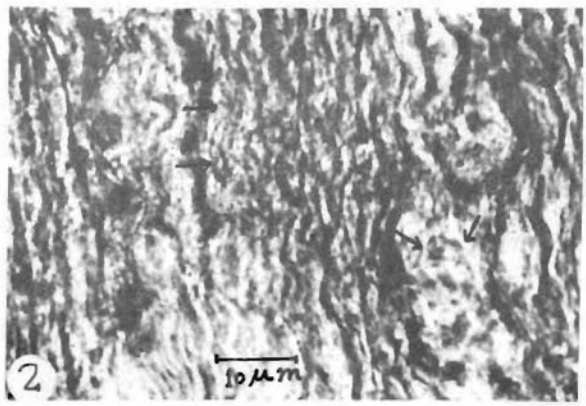

Fig. 2 : Sarin exposed showing light degeneration and swollen axons, which are surronded by macrophages (arrow). Luxol Fast blue & Holm's silver nitrate method. Scale bar = 10 µm.

DISCCUSION

In the present study mipafox induced delayed neurotoxic symptoms (severe ataxia) in hens (Table I). However, when treated with a single dose also caused production of clinical symptoms and inhibition of NTE in hens (2, 3). Sarin which is a nerve gas has been reported to inhibit NTE *in vitro* but NTE inhibition was below the threshold level *in vivo* (10). Moreover, sarin can induce OPIDN in hens after a single massive dose (11). In the present study sarin after repeated exposure to hens produced moderate ataxia and significantly inhibited NTE in all the tissues (Table I) suggested that it can induce OPIDN in hens after repeated exposure. Massive parathion exposure to human can induce neurotoxic symptoms (12). However, other reports indicated that single exposure to parathion did not induce OPIDN in hens (4). In the present study even after repeated exposure, parathion is devoid of producing OPIDN in hens. The results of the present study show that the NTE inhibition by mipafox and sarin is within the threshold level (Table I) and the inhibition of NTE in the brain and platelets is similar thus support the platelet NTE as a peripheral marker for OPIDN (5). There is a significant correlation between NTE inhibition and the lesions in the spinal cord of hens treated with mipafox or sarin (Table I). This relationship has been reported to occur in animals after single exposure to neurotoxic OP compounds (2, 11). The noticiable feature of the present study is the correlation of neurotoxic symptoms, NTE inhibition and spinal cord lesions in hens after repeated administration of OP compounds. It is concluded that mipafox and sarin are most and moderate delayed neurotoxic compounds to hens after repeated exposure. However, parathion is not a delayed neurotoxic.

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