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Assessing Delayed Neurotoxicity in Rodents after Nerve Gas Exposure

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ABSTRACT

Delayed neurotoxicity of an organophosphorus nerve gas, Sarin (a chemical warfare agent) following repeated inhalation exposure in rats and mice, was studied by behavioural, biochemical and histopathological analyses. Rats exposed to Sarin aerosols (12.5 mg/m^3 for 20 min) daily for ten days did not exhibit any clinical sign of delayed neurotoxicity. Neurotoxic esterase (NTE) activity in the brain, spinal cord and platelets was significantly inhibited, but the inhibition was below the threshold. Histopathological examination of spinal cord did not show any axonal degeneration. Mice exposed to Sarin aerosols (5 mg/m^3 for 20 min) daily for ten days developed mild ataxia and muscular weakness of the hind limb on 14th day after the start of exposure. NTE activity was significantly inhibited in brain, spinal cord and platelets. Histopathology of spinal cord showed focal axonal degeneration. Acetyl-choline esterase activity in the platelets of both the animals was significantly inhibited. We conclude that mice are sensitive to delayed neurotoxicity induced by repeated exposure to Sarin whereas rats are insensitive.

1. INTRODUCTION

The delayed type of neurotoxic syndrome caused by certain organophosphorus (OP) compounds in humans, birds and other animals has been known as organophosphorus ester-induced delayed neurotoxicity (OPIDN)[1-3]. This syndrome is characterized by a delay period of 4–21 days after exposure to OP compounds before clinical symptoms-ataxia and paralysis-are manifested [4-6]. The biochemical target for OPIDN is an enzyme, neurotoxic esterase (NTE). However, axonal degeneration followed by demyelination are the histopathological changes[4,5]. Hens were quickly established as the animal model most useful for assessing the OPIDN[4,5]. However, recent evidences have shown that although rodents (more commonly used laboratory animals) are less sensitive than hens, they develop delayed neurotoxicity after exposure to OP compounds[7-9]. Sarin, an organophosphorus nerve gas, is frequently used as a chemical warfare agent after the second world war[10]. Inhalation exposure of animals to this chemical, besides producing acute lethal effects, may cause delayed neurotoxic effects. The aim of our study was to assess the delayed neurotoxicity caused by inhalation exposure to Sarin in two species of rodents by biochemical, behavioural and histopathological analyses.

2. MATERIALS AND METHODS

2.1 Chemicals

N,N-diisopropyl fluorophosphoramidate (Mipafox), *O,O*-diethyl-*O*-4-nitrophenyl phosphate (Paraoxon), Isopropylmethyl phosphofluoridate (Sarin) and phenyl valerate were synthesized in our synthetic chemistry division and their purities were checked before experiment.

2.2 Animals

Female albino rats of Wistar strain (150-160 g) and female Swiss mice (20-25 g) were used in the study Rats as well as mice were divided into two groups of seven animals each and provided food and water *ad libitum*.

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2.3 Inhalation Exposure

The animals (rats or mice) in group 1 were exposed to fresh filtered air for 20 min daily for ten days in a dynamically operated, whole-body exposure chamber of 50-litre capacity and served as controls while those in group 2 were exposed to Sarin aerosols.

Twenty-four and 10 of Sarin for rats and mice respectively were dissolved in 10 ml of distilled water in a graduated tube and the solution was delivered at the rate of 7.2 ml/h to the liquid pick-up capillary of the air blast nebulizer using a syringe infusion pump (Braun, Germany). Filtered air was passed (26 litres/min) through central capillary at a pressure of 1.0 kg/cm^2. The nominal concentration of Sarin was 12.5 mg/m^3 for rats and 5 mg/m^3 for mice. Rats were exposed to Sarin aerosols @ 12.5 mg/m^3 (= 0.25 LC_{50}) and mice to @ 5 mg/m^3 (= 0.25 LC_{50}) for 20 min daily for ten days.

2.4 Behavioural analysis

After exposing the animals to Sarin, we observed any delayed neurotoxic symptoms and analysed them on a four-point scale as described by Sprague *et al*[11].

2.5 Biochemical analysis

The animals were sacrificed on 14th day after the start of the exposure and blood was collected in tubes containing 0.1 *M* citrate buffer, pH 7.4. The platelets were isolated from the blood as described previously[12]. NTE activity in the brain, spinal cord and platelets was assayed by the method of Johnson[13], and acetyl choline esterase (AChE) activity was determined as described by Ellman *et al*[14]. Protein was estimated by the method of Lowry *et al*[15].

2.6 Histopathological analysis

Specimens of the spinal cord were fixed in neutral phosphate buffered formalin and embedded in paraffin. Paraffin sections (8μm) were stained with hematoxylin and eosin with luxol fast blue and Holm's silver stain and gold chloride[16] for examination under light microscope.

2.7 Statistical analysis

Data were analysed statistically using student's 't' test

3. RESULTS AND DISCUSSION

The results of our study demonstrated that repeated exposure of mice to Sarin resulted in mild ataxia and muscular weakness of the hind limb, but rats were resistant to Sarin-induced delayed neurotoxic symptoms on 14th day after exposure (Table 1). The resistance may be due to species variabilities in the target tissue (e.g. central and peripheral nerves) as demonstrated in other reports also[2,17]. The NTE activity in brain, spinal cord and platelets and AChE activity in platelets of rats and mice were significantly inhibited after exposure to Sarin (Table 1). It has been previously reported that NTE inhibition to the extent of 70-90 per cent in the case of a single exposure and to the extent of 45-65 per cent in the case of multiple exposure are necessary to initiate the delayed neurotoxicity and these have been predictive of OPIDN in a variety of conventional test species[5,18]. Our results indicate that NTE inhibition in mice is within the threshold; however, in rats it is below the threshold, suggesting that mice are more sensitive to OPIDN in comparison to rats. The inhibition of NTE in the brain and platelets is of the same order of magnitude and supports the suggestion that platelet NTE is a peripheral biochemical marker for OPIDN[12].

Table 1. Effect of Sarin on neurotoxic and acetylcholine esterases and on behavioural changes (ataxia) in rats and mice

Animals	NTE[a]			AChE[b]	Ataxia
	Brain	Spinal Cord	Platelets	Platelets	
Rats					
Control	880.50 ±18.60	320.40 ±12.40	6.24 ±0.26	174.20 ±7.00	
	569.20* ±14.20	259.80* ±10.50	4.17* ±0.30	50.10* ±3.50	
Mice					
Control	680.50 ±12.60	258.60 ±10.25	4.01 ±0.20	164.10 ±6.05	
Sarin	277.00* ±11.40	135.80* ±9.50	1.80* ±0.22	38.65* ±5.02	

Animals were sacrificed on 14th day after exposure. Values are mean ±S.E.

a = *n* moles of phenyl valerate hydrolysed/min/g tissue or mg of protein.

b – *h* moles of acetylthiocholine hydrolysed/min/mg protein.

+ = mild ataxia; – = no ataxia

* = Significantly different from the corresponding values of control ($p < 0.001$).

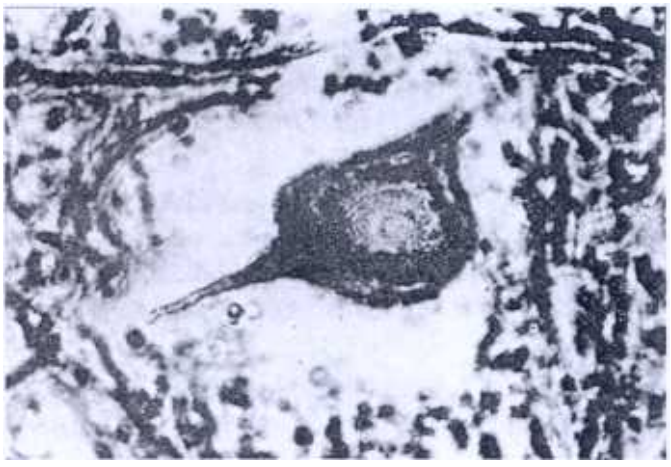

Figure 1. Cross-section of spinal cord of rat and mice showing normal neurons (H & E × 200).

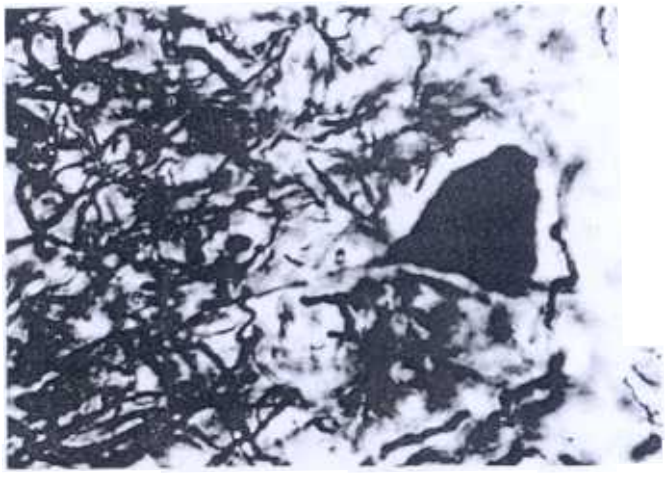

Figure 2. Spinal cord of Sarin-exposed mouse showing degenerating axon (H & E × 200).

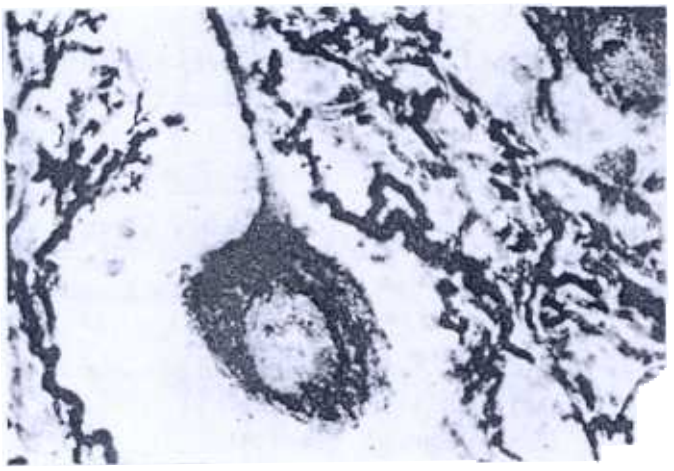

Figure 3. Spinal cord of rat following Sarin exposure. Note the neurons are normal in appearance (H & E × 200).

Histopathological examination of sections of spinal cord of control rats and mice showed normal neurons with axons (Fig. 1). The spinal cord of mice exposed to Sarin aerosols showed focal degeneration of axons of the lateral columns of the cord (Fig. 2), whereas the spinal cord of the rats exposed to Sarin did not show axonal degeneration except swollen axons without fragmentation and loss of myelin at a few places (Fig. 3). The degeneration densities were light and moderate in mice exposed to Sarin and no significant effects were observed in rats. There is a significant correlation between NTE inhibition and lesions in the spinal cord of mice exposed to Sarin (Table 1 & Fig. 2). A similar correlation was reported in the hens, which are also more susceptible to OPIDN than mice[4,5,19]. The lack of any correlation between OPIDN-induced NTE inhibition and the spinal cord lesions in rats suggests that mice are a better model than rats[7–9]. Our study demonstrated that repeated inhalation of Sarin-induced delayed neurotoxicity in mice but not in rats. We suggest that for assessing OPIDN in laboratory rodents, behavioural, biochemical and histopathological tests may be employed.

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