

STUDY OF ACUTE DELAYED NEUROTOXICITY OF TRIORTHOCRESYLPHOSPHATE (TOCP) OF SCIATIC NERVE BY LIGHT MICROSCOPE IN ADULT HEN

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ABSTRACT

Acute delayed neurotoxicity of TOCP (triorthocresylphosphate) as positive control for organophosphorus, light microscopy of Toluidine blue and Haematoxylin and eosin (H&E) stains showed varying degrees of degenerate nerve fibers associated with clumps of degenerate myelin also areas of demyelination of nerve fibers. The above changes were supported by clinical findings of treated hens with in-coordination, ataxia and paralysis.

KEYWORDS: TOCP, Light Microscope, Sciatic Nerve and Adult Hen.

INTRODUCTION

Most organophosphorus esters are direct inhibitors or are rapidly converted to inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7) [1-3]. Some of these compounds produce a more persistent effect: delayed neurotoxicity. In humans, organophosphorus ester-induced delayed neurotoxicity (OPIDN) results in a flaccid paresis which develops distally in the legs and spreads to the hands and thighs. In the later stages, symptoms of spinal cord injury such as spasticity and ataxia become evident as the symptoms of peripheral neuropathy recede. OPIDN has the following features: Most of the delayed neurotoxic organophosphorus esters are AChE (acetylcholinesterase) inhibitors, but not all anticholinesterase compounds produce delayed neurotoxicity. There is a latent period after the administration of a single dose and before the onset of clinical signs, which ranges between 6 and 14 days. Cellular damage is seen in the sciatic, peroneal, and tibial nerves; spinal cord; and medulla, but not in higher brain. Onset of lesions begins at the distal part of long fibers and of large diameter peripheral nerves. The lesions are characterized by the degeneration of the axons with subsequent secondary degeneration of myelin. Not all animal species are susceptible to OPIDN; humans are believed to be among the most sensitive species. Species sensitivity appears to be related to age; that is young chicks are insensitive. The biological and pathological effects have

been well reviewed previously [4-10]. This review emphasizes the relationship between the chemical structure of organophosphorus esters and their ability to induce delayed neurotoxicity. It also discusses the biochemical target as well as factors affecting the development of OPIDN. OPIDN was first recognized at the end of the nineteenth century in humans poisoned with TOCPI [11, 12]. Since then an estimated 40,000 cases of delayed neurotoxicity in humans have been documented. In the 1920s about 20,000 persons in the United States developed "Ginger-Jake" paralysis after the consumption of an extract of ginger called "Jamaica Ginger" that had been adulterated with TOCP [13-21]. Later this syndrome was recognized in Europe, South Africa, and India as a result of the deliberate or accidental use of TOCP-containing preparations [22-34]. In 1951 three persons were poisoned with a then newly developed insecticide, mipafox, and developed symptoms of delayed neurotoxicity [35]. Between 1974 and 1975 the experimental insecticide leptophos was implicated in the poisoning and paralysis of some workers in the Texas factory where it was manufactured and packaged [36, 37]. Symptoms associated with TOCP [38-42] and mipafox [35, 43] poisoning in human subjects are well documented. The clinical conditions of persons in the leptophos incident were diagnosed as multiple sclerosis, encephalitis, or psychiatric disorder [35, 37, 44]. These cases were further complicated by the possibility that some of the workers were simultaneously exposed to solvents such as toluene and n-hexane. Cases of distal neuropathy due to chronic exposure to n-hexane have been revealed [45].

MATERIALS AND METHODS

Experimental design

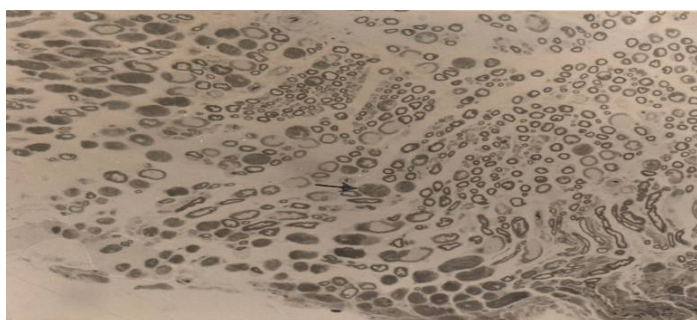
Sixty adult hens were divided in two groups, 30 untreated control and 30 dosed orally with single dose of TOCP (triorthocresylphosphate) as positive control for organophosphorus of 500 mg/kg delayed acute neurotoxicity for 21 days, clinical signs appeared after 10 days of treatment and progressed till the end of 21 days, symptoms various from in coordination, ataxia and paralysis, there were grades of those changes from 1 to 6, samples of sciatic nerve were stained with Toluidine blue and H&E stains, additional photographs of hens with varying grades of in coordination, ataxia and paralysis. Always at the end of the 21 days of acute delayed neurotoxicity of TOCP some hens survived with hardly any clinical symptoms, but those often showed microscopic light microscopic lesions.

Examination

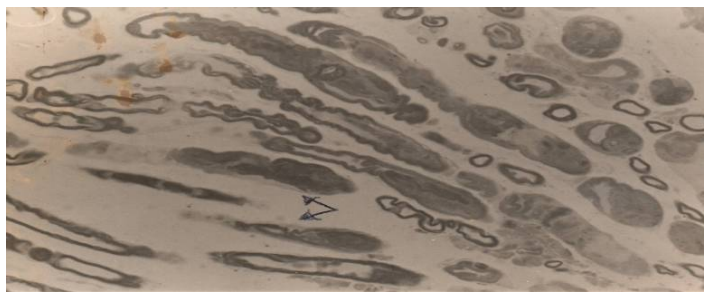
Samples for histopathological examination were taken from sciatic nerve, those were cut in pieces of 2 to 3 cubic centimeters, samples were fixed in 10% phosphate buffered formalin and left in fixative for several days, the materials were embedded in paraffin, then paraffin blocks were made and cut by microtome at 5-7 microns, passing through different concentration of alcohol for dehydration and then rehydration to remove the paraffin then sections were stained by Toluidine blue and H&E stains. then sections were examined with light microscope on different power for histopathological changes[46]

RESULTS

Light microscopy of sciatic nerve of treated adult hens of acute delayed neurotoxicity stained with Toluidine blue showed varying number of degenerate nerve fibers with degenerate myelin as clumps as in (fig1), with high magnification, longitudinal and transverse sections of nerve fibers with degenerate vacuolated nerve fibers associated with clumps of myelin as masses (fig2). Still with high magnification it demonstrates clearly the degenerate myelin as masses or clumps (fig3). In fig4, nerve fibers showing clumps of degenerate myelin. Some of the degenerate myelin appeared in myelinophages as were shown in (fig5). The same changes were seen in sections of H&E stain, (fig 6) & (fig7) on different magnification showed the same histopathological changes as Toluidine blue stain.



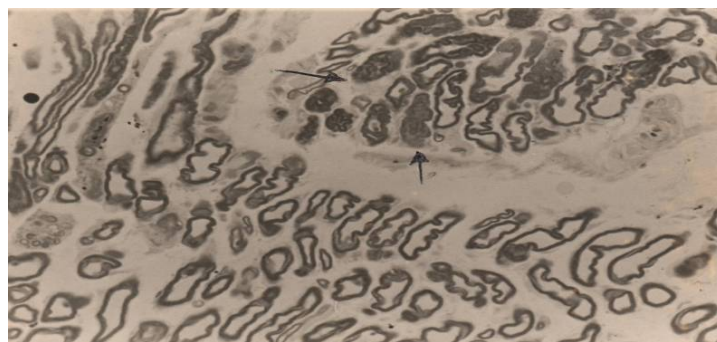
Fig(1): neurotoxicity of TOCP, sciatic nerve note various nerve fibers some of them with degeneration associated with clumps of degenerate myelin.(arrow) (10X, Toluidine blue stain, light microscope).



Fig(2): neurotoxicity of TOCP, sciatic nerve note longitudinal section of degenerate nerve fibers with clumps of degenerate myelin.(arrows) (20X, Toluidine blue stain, light microscope).



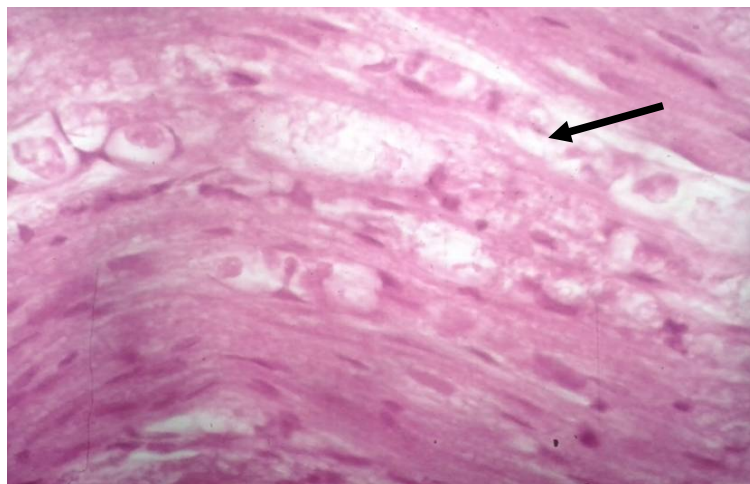
Fig(3):): neurotoxicity of TOCP, sciatic nerve note longitudinal section of degenerate nerve fibers with clumps of degenerate myelin.(arrow) (40X, Toluidine blue stain, light microscope).



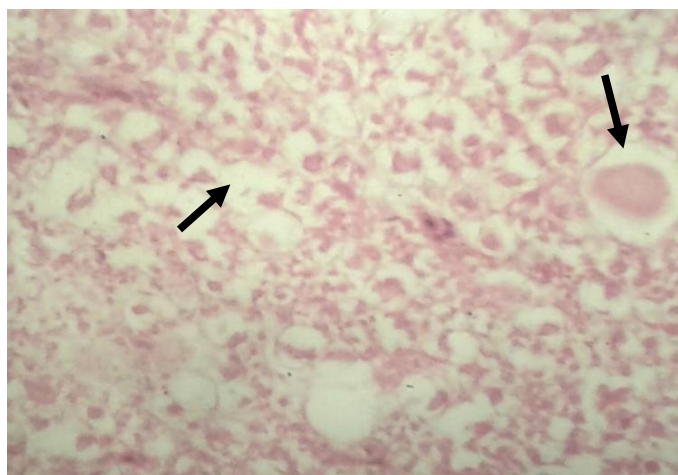
Fig(4): neurotoxicity of TOCP, sciatic nerve note fewer number of degenerate nerve fibers . (arrows) (20X, Toluidine blue stain, light microscope).



Fig(5): neurotoxicity of TOCP, sciatic nerve note some demyelinated nerve fibers associated with myelinophages. (arrows) (40X, Toluidine blue stain, light microscope).



Fig(6): neurotoxicity of TOCP, sciatic nerve note nerve fibers with degeneration and vaccuolation, some vacuoles associated with clumps of degenerate myelin. (arrow) (40X, H&E stain, light microscope).



Fig(7): neurotoxicity of TOCP, sciatic nerve note fast number of vacuolated degenerate nerve fibers, some of which associate with clumps of degenerate myelin. (arrows) (40X, H&E stain, light microscope).

DISCUSSION

The neurotoxicity of TOCP(tri-ortho-cresyl-phosphate) , with acute delayed neurotoxicity, single oral dose of 500 mg/kg with corn oil in adult hen, the age related and species related effects of TOCP neurotoxicity was discussed by[47] and this applied on the present study as it was found that adult hen the best model for delayed organophosphorus delayed neurotoxicity, even with development of the lesions affecting long tract by myelinated nerve fibers. In addition[52] discussed mechanisms of organophosphorus stressing the fact that TOCP and some other organophosphorus induced delayed neurotoxicity with clinical signs and light microscopic lesions in sciatic nerve. Delayed neurotoxicity by TOCP and some

other organophosphorus compounds were in references [47-51]. The lesions reported were in agreement with the light microscopic findings in sciatic nerve also agreed with clinical findings too, [47]review the neurotoxicity of organophosphorus and reported OPIDN due to TOCP and that agreed with our findings in spinal cord of adult hen, including the clinical signs, but one thing that the lesions in sciatic nerve in acute delayed neurotoxicity more severe than those of spinal cord, some which only recede in subchronic neurotoxicity of peripheral nerve will be less and that of spinal cord will progress and that will be in OPIDN.

[11-44],the history of organophosphorus with clinical signs and pathological neural lesions were in agreement with what we found clinically and on base of light microscopy of spinal cord. In spinal cord in acute neurotoxicity the changes of spinal cord were less than sciatic nerve but in ninety days neurotoxicity study, the changes of spinal cord more prominent than the sciatic nerve, and that means the changes of the sciatic nerve were reduced in ninety days and increased in spinal cord.

Research highlights

The present research paper on clinical and light microscopy of sciatic nerve brings new and interesting findings on neurotoxicity of organophosphorus.

Finding and policy aspects

The findings present clear and scientific changes induced by organophosphorus clinically and on base of light microscopy and open the way for research on neurotoxicity.

Justification of research

The present work is quite beneficial for safety of people and animal against neurotoxicity of organophosphorus.

Conclusion

The study concludes that organophosphorus can give clinical signs in association with light microscopic changes.

Recommendation

The present neurotoxic research showed that adult hen can be a good model for neurotoxicity clinically and on light microscopy.

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