ULTRASTRUCTURE OF NERVE AND MUSCLE FIBERS FOLLOWING ORGANOPHOSPHATE POISONING

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SUMMARY: This study indicates the ultrastructural changes of sural nerve and gastrocnemius muscle biopsies of a patient poisoned as a result of organophosphate (Tamaron) intake for suicide attempt. Studying the micro-morphology relative decrease of axon diameter which was a result of excessive thickening of the myelin sheaths, and spiral invagination deep into the axon was apparent especially in thick axons was observed. The regular concentric layers of the myelin sheath were destroyed. There was a consistent increase in nuclear heterochromatin, accumulation of glycogen, irregular vacuolization and membranous structures in cytoplasm of Schwann cells. Disarrangement of Sarcomer and myofibrils was apparent in severe degenerated areas. In some micrographs, intact and degenerated areas were coexistent. Extremely thickened capillary basal lamina was remarkable where there was disorganization of muscle fibers. It is concluded that the muscle degeneration in organophosphate poisoning can not be attributed solely to the axonal degeneration but also to the direct toxic effect of the compound.

Key Words: Organophosphate poisoning, ultrastructure, nerve fiber, muscle fiber.

INTRODUCTION

Accidental organophosphate poisoning commonly occurs in the agricultural area of Turkey as organophosphate insecticides are widely used in the Çukurova region. Suicide attempts with organophosphates are therefore more common causes of contamination (11, 16). At the end the patient reveals a cholinergic crisis which may be severe enough to make the person unconscious and the diagnosis is confirmed as the symptoms are controlled by atropine therapy. Later polyneuropathological symptoms develop as a complication (9, 18, 20). However, here have been a few ultrastructural analyses of both the nerve and muscle affected by organophosphate insecticides (1, 18).

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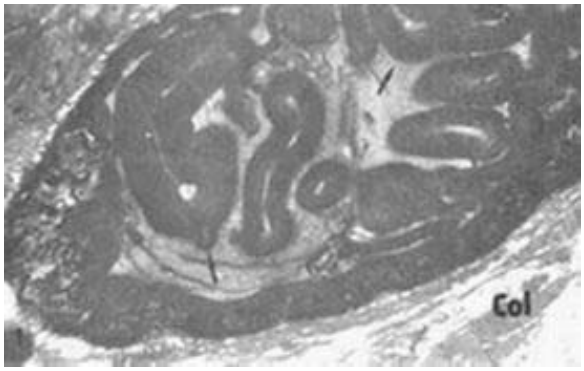
MATERIAL AND METHOD

A twenty year old man was admitted to the hospital in an unconscious state, having ingested about 20 ml of Tamaron (dimethyl phosphoramidothiate) in a suicide attempt. In the physical examination, the presence of miosis, hyper-salivation and excessive sweating confirmed the diagnosis of organophosphate poisoning. After the beginning of atropine therapy, the symptoms receded gradually and four days later he recovered completely at which time he was discharged from the hospital. After fifteen days, he reapplied to the hospital, complaining of limb pain and weakness. He was conscious and cooperative, his temperature was 36,5°C, heart rate was 100 beats/min and arterial blood pressure was 130/80 mmHg. Neurological examination revealed weakness of the distal limb muscles, particularly in lower limbs; the ankle reflexes were absent but the other reflexes were normal. There was no cranial nerve palsy, no sensory impairment and the plantar responses were flexor.

Figure 1: The nerve fiber exhibits a marked degeneration (asterix) and invaginations (arrows) of myelin sheath. Collagen (Col). Sural nerve biopsy. X 12.400.



The following investigations showed no significant abnormalities: erythrocyte sedimentation rate, hemoglobin, white cell count, fasting blood sugar, blood urea nitrogen, serum electrolytes, serum glutamic oxaloacetic transaminase, alkaline phosphatase, serum proteins, calcium, phosphate, serum uric acid, serum creatinine, complete cerebrospinal fluid and urine examinations, radiography of chest and electrocardiogram.

Sural nerve and gastrocnemius muscle biopsies were performed and were immediately placed in 5% glutaraldehyde buffered at pH 7.4 with Millonig phosphate buffer for 4 hours. The tissue pieces were subsequently fixed in 1% OsO_4 for 2 hours and then dehydrated in graded ethanol solutions, embedded in araldite, and processed for electron microscopy using conventional methods.

RESULTS

Electron microscopic observation on the sural nerve biopsy revealed excessive thickening and obvious degeneration of myelin sheath which was particularly noted in inner lamellae. The regular concentric lamellation of the myelin sheath had disappeared and irregular spaces were formed due to separation of lamellae from each other. The diameter of axonal spaces was diminished by the thickening and spiral invagination of the myelin sheath deep in to the axons (Figures 1 and 3). In most of the micrographs, nerve fibers with larger diameter were closely packed and tightly adherent to each other. Degeneration was more evident in these axons than the thinner ones (Figure 2). An increase in nuclear

Figure 2: Thicker nerve fibers are seen closely packed and tightly adhered each other (thick arrows). Degeneration was less evident in thinner axons (thin arrows). Collagen (Col). Sural nerve biopsy. X 12.400.

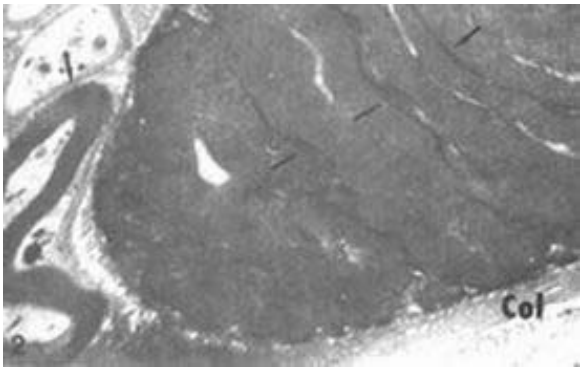
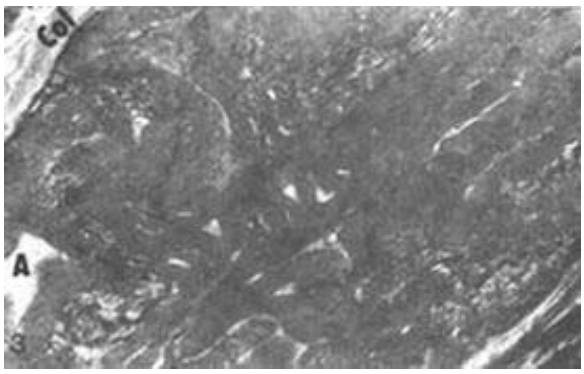


Figure 3: Axonal spaces (A) are extremely diminished by the excessive thickening of myelin sheath. Collagen (Col). Sural nerve biopsy. X 16.100.



heterochromatin, membranous structures, irregular vacuoles and accumulation of glycogen particles in cytoplasm of Schwann cells were seen common findings (Figure 4). Extensive endoneurial deposition of collagen fibrils arranged as irregular bundles were observed in degenerated areas (Figures 1, 2, 3 and 4).

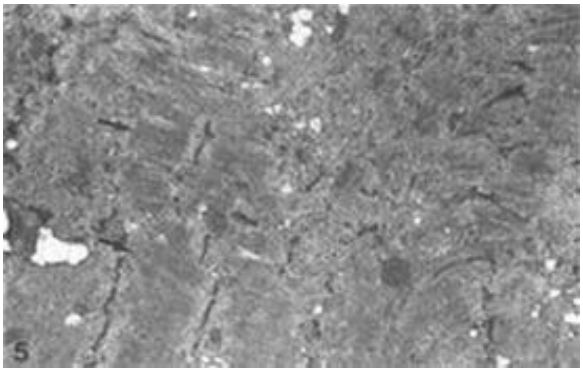
Gastrocnemius muscle biopsy exhibited disruption of the regular arrangement of sarcomers (Figure 5) and myofibrils which were in the form of irregular bundles (Figure 6). In these muscle cells, typical myofibrilar cross-banding had entirely disappeared. Furthermore disorganization and fragmentation of myofilaments in vicinity of Z-bands were noted. In some muscle cells, intact and degenerated areas existed. There were also deposition of glycogen particles beneath the sarcolemma and among the myofibrils. In these foci, variously sized vacuoles which were not bound by a membrane were seen (Figures 7 and 8). Thickening of the capillary basal lamina was a striking feature in adjacent areas of degenerated muscle fibers (Figure 8).

DISCUSSION

Organophosphate insecticides including Tamaron are widely used in Turkey for agricultural purpose. Tamaron's main ingredient is methamidophos (O, S dimethyl phosphoramidothiate) (15). In our case, organophosphate intoxication as a result of Tamaron ingestion, in an attempt at suicide resulted in cholinergic crisis. Two weeks following the disappearance of cholinergic symptoms, the initial signs of delayed neuropathy became apparent.

Figure 4: Degenerated axons (A) are surrounded by Schwann cells cytoplasm (Sc) which contains membranous structures (M), irregular vacuoles (V) and excessive glycogen particles. Note the presence of excessive collagen. Nucleus (N). Sural nerve biopsy. X 16.100.

Figure 5: A muscle cell with disrupted regular arrangement of sarcomers (arrows) are seen. Gastrocnemius muscle biopsy. X 10.100.



The acute toxic effects of organophosphates are well known (1,9,11,13,15,16,20). As indicated by some workers, prominent toxic effects of these compounds exhibit in the following 24 hours after the ingestion and the symptoms can be controlled by aggressive atropine therapy (2). Organophosphates act as irreversible inhibitors of carboxylic esterases, the most significant of which is cholinesterase and effect by binding the active sites of the enzymes. As a consequence of stimulation by increased amounts of acetylcholine at the neuromuscular junction, neurotoxicity develops (1,7,11,16,17). It is reported that organophosphates produce a characteristic pattern of axonal degeneration, involving selectively the distal portions of the long and large axons. This axonal degeneration is expressed clinically as a symmetric, distal, sensorimotor polyneuropathy, so-called dying-back neuropathy (3,4,14). Spreading of focal and non-terminal axonal degeneration in a somatofugal direction to involve the entire distal axon is reported (4).

In most of the micrographs, numerous degenerated nerve fibers, unstructured myelin sheaths and extremely diminished axon diameter by the spiral invagination of the myelin sheath were observed. Structural abnormalities of the nerve fibers due to the high amount of acetylcholine accumulation of the terminal axons have been observed (7,16). Particular effect of organophosphates on longer and larger axons has also been reported (3,4). In our micrographs, degeneration was apparent in especially larger axons. The structural changes characterized by an increase in nuclear heterochromatin, cytoplasmic glycogen accumulation, membranous structures and vacuoles of various size, in the Schwann cells express a probable functional abnor-

Figure 6: Irregular bundles of myofibrils (arrows) are seen in sarcoplasm. Gastrocnemius muscle biopsy. X 8.100.

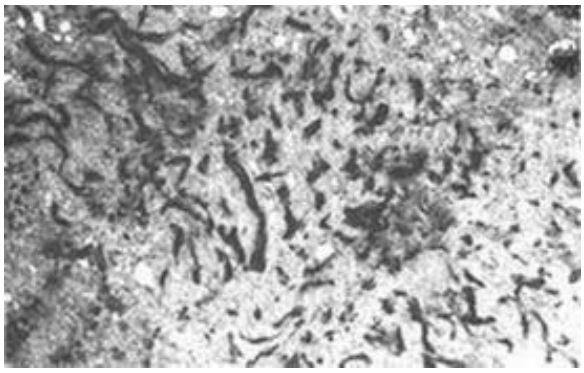
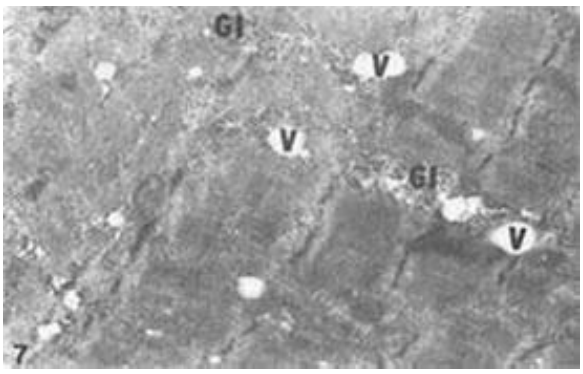


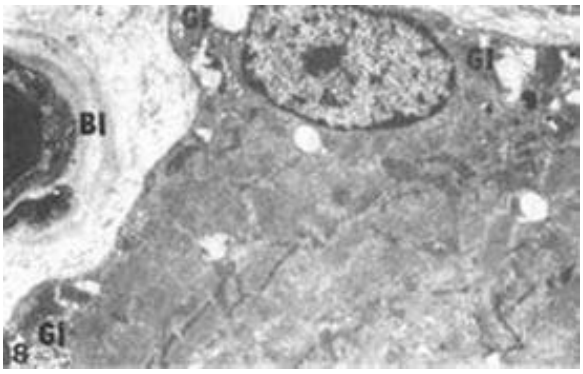
Figure 7: Among the myofibers, deposition of glycogen particles (GI) and variously sized vacuoles (V) are seen. Gastrocnemius muscle biopsy. X 12.400.



mality. Bouldin and Cavanagh (3) constructed the paranodal demyelination by the direct toxic effect of disopropylflurophosphate (DFP), to the Schwann cells. Most of the organophosphates are lipophilic substances (17) and myelin is largely composed of lipid. Borowitz (2) showed that fenthion (a lipophilic organophosphate) accumulates mostly in adipose tissue and by being released gradually, inactivates the existing and newly synthesized cholinesterase enzymes. Tamaron, probably, causes abnormal myelin sheath production by binding to the myelin sheath. Organophosphate compounds effect mainly the larger axons structure and function, probably due to the high lipid content of their myelin sheath.

As a result of the neuromuscular transmission blockade, paralysis of the proximal extremities, flexor muscles of the neck and respiratory muscles occurs as well (13). Ludomirsky *et al.* (7) reported the toxic effects of organophosphates on the heart muscle; malignant ventricular arrhythmia and prolonged Q-T interval in ECG. In chronic organophosphate toxicity, myophagocytosis, endomysial fibrosis, severely atrophic myofibers and disruption of the organized arrangement of myofilaments in some of the myofibrils have been reported as common features. The authors verified that chronic exposure of the motor end-plate to increased levels of a acetylcholine caused the observed necrosis of the muscle (1). Vasilescu *et al.* (18) reported that the abnormalities of the muscles are mostly apparent in the Z-band. It can be accepted that pathologic changes in striated muscles in organophosphate in toxicity are secondary myopathic signs due to denervation (1,10). In neurogenic myopathy, it is confirmed that in spite of focal

Figure 8: Accumulation of glycogen particles (GI) beneath the sarcolemma and excessive thickening of capillary basal lamina (BI) are seen. Gastrocnemius muscle biopsy. X 8.100.



degeneration and progressive reduction in the myofibril diameter, the sarcomer is completely intact (6). The features of disorganization of Z-bands in myofilaments as well as focal necrosis as characteristic of neurogenic myopathy were striking in our case. Adjacent areas exhibited complete disarrangement of the sarcomer, excessive cytoplasmic glycogen accumulation, which is one of the sings of the dysfunction, in the myofibrils and thickening of the capillary basal lamina. These pathologic changes can be attributed to both the axonal degeneration and Tamaron's direct toxicity. The function of the basal lamina as a selective barrier in macromolecule exchange is well known. As a matter of fact, thickening of capillary basal lamina providing the protection of the tissues from noxious agents has been observed in some organ studies (5,8,19). As a result, it can be concluded that the muscle degenerations in organophosphate insecticide intoxication can not be attributed only to the axonal degeneration, but to the direct toxic effects of these compounds as well.

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