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The Effects of Pentoxifylline in Experimental Nerve Injury

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Abstract: Several studies have suggested that pentoxifylline, a theophylline-related drug that decreases erytrhocyte stiffness, may be useful in treatment of ischaemia reperfusion injuries of brain, liver and small intestine in animal experiments. Fourteen Wistar-Albino rats weighing between 180-250 g were used for the experiment and divided equally to two groups. Before the injury, electroneuromyographic (ENMG) data were recorded from right and left sciatic nerves. Right sciatic nerves of the seven rats of group 1 were crushed with a clip. Same lesions were performed on seven rats of group 2 and also pentoxifylline (50mg/kg/day) was applied intraperitoneally once in a day for 21 days.

Immediately after the third week of the operation, electrophysiological studies were performed in the both groups again. In the treated group latencies of compound muscle action potentials (CMAP) treated with the pentoxifylline were found shorter than the untreated group, but this difference was not statistically significant. Whereas, in the animals treated with pentoxifylline, amplitudes of the CMAP recordings were higher than the untreated group and the difference was statistically significant.

These results suggested that pentoxifylline has a positive effect on axon regeneration but no significant improving effect on segmental remyelination.

Key Words: Experimental nerve injury, pentoxifylline, sciatic nerve

Introduction

Peripheral nerve injury was always been a critical clinical problem in human history, for this reason a variety of experimental and clinical studies has conducted by the researchers to seek new treatment methods of these lesions. Complete lesions, such as neurotmesis can be treated by surgical methods but axonotmetic nerve injury is treatable with conservative medical treatment methods. Studies in the field of peripheral nerve injury were began in 1795 and especially accelerated during the World War I and II on account of war injuries. Today, pharmacological agents are used in the treatment of these lesions, including non-steroid anti-inflammatory agents, steroids (1), nerve growth factors (2,3), Rheomacrodex (4), thyroid hormones (5), growth hormone (6), ACTH (3,7) and insulin-like-peptide (6,8).

In this study, we used pentoxifylline, a phosphodiesterase inhibitor, for its rheologic properties and discussed its effects on the nerve injury model, electrophysiologically.

Materials and Methods

This study had been conducted in the Institute of Experimental Surgery of Karadeniz Technical University in 1998. Fourteen Wistar-Albino rats were used and divided into equal two groups regardless of sex, seven in control group and seven in the treated with pentoxifylline, the weights of animals were between 180 and 270 grams.

Animals were anesthetized with 70-mg/kg ketamine-HCl and positioned on wooden frame, designed for experimental studies. Before injuries were performed ENMG recordings were taken in all groups. Then the right sciatic nerves of the animals were explored under a surgical microscope. Then, sciatic nerve was clipped by mini aneurism clip, (Yasargil FE693 Aneurism Clip, Aesculap) for 90 seconds to form crush injury and then removed. In all animals, post injury ENMG recordings were performed.

Fifty-mgr/kg/day pentoxifylline (Trental, Hoechst) was given intraperitoneally to all experimental groups in

the first day of the injury, once a day, and repeated for in every twenty-four hours for twenty-one days. After the twenty-four hours from the last drug application, ENMG recordings of all animals were taken again and evaluated.

Electrophysiological Evaluation Method

Electrophysiological recordings were performed in a quiet room with an ambient temperature between 22-23 ºC, using Medelec Premier ENMG apparatus. Wraparound ground electrode (54482T) placed on the animal's thorax externally. Modified surface electrodes (Medronic, 9013L0202, 4 mm in diamater) were used to record CMAPs. The hind limbs of the rats shaved accordingly. Using electrode gel, the cathode was located on tibialis anterior muscle, 1.5 cm distal to injury zone, and the reference electrode located on the tendon of the muscle. Adjusting distance between cathode and anode to 1 cm, the sciatic nerve was stimulated at the sciatic notch 1.5 cm proximal to injury zone, by two monopolar needle electrodes. Supra-maximal pulses, 0.05 ms in duration, were delivered. Filter settings were 0.1-3 KHz; sweep speed 2 ms/div, and sensitivity 2-10mV/div. The latency was measured to the onset of the CMAP, thus giving a measure of the conduction velocity of the fastest fiber. Baseline to peak amplitudes, which show the numbers of fibers activated by nerve stimulation, were also measured.

Electrophysiological data's were analyzed using Mann-Whitney U test

Results

ENMG Findings:

The latencies of the CMAP recordings of the treated animals were shorter than the untreated group, but this difference was not statistically significant ($p>0.05$). Whereas, in the animals treated with pentoxifylline, Amplitudes of CMAPs were higher than the untreated group and the difference was statistically significant ($p<0.01$) as ENMG findings were summarized in table 1.

Table 1. ENMG Results.

		On the third week evaluation	
	Preinjury n=14	Injured n=7	Injured +treated n=7
Latency (ms)	1.16±0.15	2.07±0.52	1.84±0.48
Amplitude (mV)	16.35±2.87	2.35±1.73	6.12±0.52*

* = On the third week evaluation mean amplitude of CMAPs were significantly higher in pentoxifylline treated group versus untreated group.

Discussion

Pentoxifylline is a phosphodiesterase inhibitor with rheologic properties along with the effects of decreasing the viscosity of the blood. Due to it's phosphodiesterase inhibition effect, cyclic adenosine monophosphate (cAMP) levels increase in erythrocytes, endothelium, and surrounding tissues (9). In addition to this rheologic effects of pentoxifylline, it has also immunomodulatory properties which produces vascular endothelial stabilization and autoimmune inhibition, especially significant in the treatment of neuritis (10). It has also been used in clinical and experimental studies of Bell's palsy (4,11,12), carpal tunnel syndrome (13), optic nerve's circulatory disorders (14), cerebrovascular accidents and some microvascular disorders (15, 16, 17, 18).

Functional integrity of peripheral nerves primarily depends on the sufficient blood supply like other tissues. Acute ischemic lesions of the peripheral nerves can be reversible but severe and prolonged ischemic process of the nerves can lead to extensive axon injury along with Wallerian degeneration (19). The severity of these lesions depends on the duration and the intensity of ischaemia and compression. Vasa nervorum can also be affected by trauma, diabetes and by some metabolites. These factors can lead to ischemic neuropathy (20,21,22) and reperfusion injury during peripheral nerve injury (23). Ischaemia leads an insufficient ATP supply when ATP consumption in all damaged tissues increase. Anaerobic metabolic process levels increase in such process and eventually hypoxanthine levels also increase in ischemic tissues. In the reperfusion phase of ischaemia superoxide radical levels and hydrogen peroxide levels increase. These can lead to the damage of the tissues (18).

Pentoxifylline had been used in some experimental and clinical studies especially to restore the normal function of the vasa vasorum (22,24). Although pentoxifylline has no effect on vascular tonus, it contributes the amolioration of the microcirculation

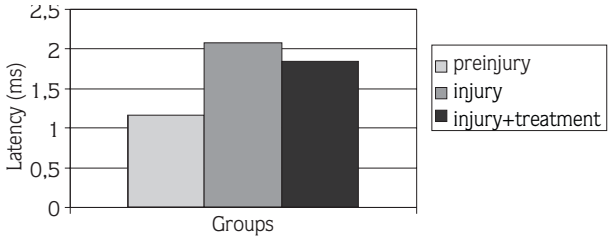

Figure 1. Mean latency values in groups. Latency were significantly shorter in preinjury group, but there were no significant differences between injured and injured+untreated groups.

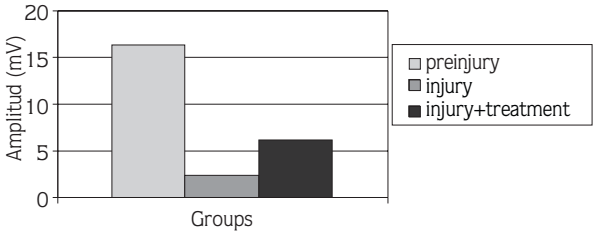

Figure 2. Mean CMAP amplitudes in groups. Amplitudes were significantly higher in preinjury group and there were significant differences between injured and injured+treated groups.

regulation and intovascular cell dynamics (5,18). Studies showed that pentoxifylline decreases peripheral nerve injury with preventing free radical production by neutrophil inhibitory effect, and also decreasing the levels of cytokines, malondialdehyde and myeloperoxidase (18). Pentoxifylline is also known to increase whole blood filtration rate, and recent evidences suggests that pentoxifylline increases the filtration rate of polymorphonuclear leucocytes in the tissues (25). Also deformability of neutrophils increased when they treated with pentoxifylline. These results have been consistent with the hypothesis of the beneficial effect of pentoxifylline on microvascular perfusion is partly due to an inhibition of polymorphonuclear cell stiffness and activation (25).

Aim of this study was testing of these beneficial effects of pentoxifylline in the peripheral crush injury which causes axon degeneration, myelin destruction and myelin destruction with nerve ischemia (26). In the evaluation of peripheral nerve regeneration, ENMG recordings revealed important information in the studies of crush injury. ENMG recordings are very important in this issue because it confirms evidences of regeneration weeks or months before motor functions improvements can be observed. Latency and amplitude of CMAP are the principle criterion has to be evaluated. Amplitude is directly correlated with active neuron number and latency is a reliable indicator of demyelination after the injury (27).

Previous studies proved that intraperitoneal pentoxifylline treatment for three weeks is adequate to evaluate its effects by ENMG (28,29). Pre and post injury evaluations by both histological and ENMG findings demonstrated that the lesion produced by the clip-compression was in grade II and III, according to of Sunderland's classification (30).

In our study, we found a statistically significant difference for amplitudes between injured and injured + treated groups. This difference demonstrates that axon regeneration was augmented by pentoxifylline treatment. There was no statistically significant difference for latency. This result demonstrates that pentoxifylline has no significant improving effect on segmental remyelination.

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