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The Contribution of Electrostimulation on Nerve Regeneration in Rabbits with Experimentally Induced Sciatic Nerve Injury

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Abstract: The purpose of the study was to investigate the effect of electrical stimulation on nerve regeneration in sciatic crush injury model in rabbits. Sixteen New Zealand male rabbits, allocated into two equal groups were used in the study. All rabbits were anaesthetized, their sciatic nerves were exposed and crush injury was induced with Bulldog clamps. Distal motor nerve latency, Compound Muscle Action Potential recorded by the distal part of crush point (dCMAP), Compound Muscle Action Potential recorded by the proximal part of crush point (pCMAP) was recorded and Nerve Conduction Velocity (NCV) was estimated before and just after the crush injury. Electrical stimulation was performed for 90 times every day for 3 weeks. At the 21st day all rabbits were anaesthetized and their sciatic nerves were exposed and the same electrophysiological examination was carried out respectively. Distal motor nerve latency, dCMAP, pCMAP and NCV were not significantly different between groups ($p>0.05$). In conclusion, our study did not support the idea that was about performing electrical stimulation to injured nerves could increase regeneration. However, the higher amplitude of dCMAP in the experiment group at the end of the study can be accepted as a positive point for electrostimulation on nerve injury.

Key words: Sciatic nerve injury, electrical stimulation, electrophysiology, rabbit, regeneration, electrostimulation

INTRODUCTION

Peripheral nerve injury still remains a common clinical problem. Recovery depends on the grade or the severity of the injury. As neuropraxic grade of injury requires remyelination of axons, axonotmetic grade of injury requires not only remyelination but also axonal regeneration (Kahn, 1991; Mourad *et al*., 2001; Nelson and Currier, 1991). Muscle atrophy and change in muscle fiber composition are the main consequences of peripheral nerve injury (Maqueste *et al*., 2004). In axonotmetic lesions like crush injury, nerve fibers regenerate approximately 1 mm per day. Despite many treatment modalities, the recovery is often incomplete (Maqueste *et al*., 2004; Mourad *et al*., 2001; Pockett and Gavin, 1985). Considering the importance of time course of functional recovery, the acceleration of regeneration of the nerves and reinnervation of the muscle is essential (Leterme, 2004). In the last decades, many authors have studied the effect of physical agents like electricity, ultrasound, low power laser and magnetic field on the outcome of the healing of peripheral nerves, soft tissue and bones. There are many researches with different methods showing evidence of acceleration of regeneration of peripheral nerve after injury (Mendonca *et al*., 2003; Mourad *et al*., 2001; Pockett and Gevin, 1985).

Electrical stimulation is a physical modality, which is a means of providing active exercise to muscles where there is lack of voluntary contraction. If the muscle is denervated, it maintains the nutrition of the muscle promoting blood flow, decrease fibrotic changes in the muscle and retard atrophy. It can also be used to strengthen the muscles, maintain mobility, provide proprioceptive feedback and promote peripheral circulation in the innervated muscles (Bevergide and Politis, 1993; Nelson and Currier, 1991; Pocket and Gavin, 1985).

The electrical stimulation, which is routinely used in daily practice, is a non invasive treatment modality. However, its effect on peripheral nerve regeneration still remains controversial. This study was designed to evaluate the effect of electrical stimulation on the crush injury model based on the electrophysiological parameters.

MATERIALS AND METHODS

Sixteen healthy male New Zealand rabbits wereincluded in this study. All animals were kept in smooth bottomed plastic cages at 22° with 12:12 light dark cycle. Appropriate food was given under the laboratory conditions.

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Surgical procedure: The rabbits were anesthetized with xylazine hydrochlorure (5 mg kg^{-1} IM, 10% Rompun, Bayer) and ketamin hydrochloride (50 mg kg^{-1} IM, Ketalar, Parke Davis) and left leg was prepared for aseptic surgery. Sciatic nerve was exposed and from the sciatic notch to the bifurcation area. The area to be crushed was marked with knotting propylene sutures at the two cm proximal to the bifurcation.

Electrophysiological technique: Medelec Synergy (Oxford instrument) 5 channel EMG/EP machine was used for the electrophysiological studies. The stimulating electrodes were inserted 2cm below the crush point and 2 cm above the crush point as active electrode inserted epineurally and reference electrode inserted about 1 cm laterally subcutaneously. The recording electrode was inserted subcutaneously over the gastrocnemius muscle at the midpoint of its entire length and referred to its tendon subcutaneously. Both stimulation and recording procedures were performed with monopolar needle electrode. The sciatic nerve was stimulated from distal and proximal of the crush area and recording was performed at gastrocnemius muscle. Frequency limits for recording were 10 Hz-2 kHz, sweep speed was 10 msecs. A rectangular, 0.1 ms duration stimulus at supramaximal intensity were used and at least 3 consecutive, repeatable CMAP were adjusted. The needle pricking points for gastrocnemius were marked with permanent Chinese ink for the second evaluation. The nerve was crushed with standard bulldog clamp for 3 min and the operation area was closed in a routine manner. The CMAP amplitude was measured as peak to peak and the latency was measured as onset latency and NCV was estimated as the distance division to the time.

Electrical stimulation: The rabbits were divided into control (n = 8) and experiment (n = 8) groups at the second day. Electrical stimulation was carried out with stimulation of sciatic nerve from sciatic notch by the pulsed Galvanic current and visible contraction of gastrocnemius muscle was maintained by the bipolar technique with 1×1 cm^2 probe (Petaş, Turkey). Two electrodes were attached on the gastrocnemius muscle 1cm apart. In the experiment group, the gastrocnemius muscle was stimulated with pulsed current with a phase duration of 100 milliseconds, 30-50 pulses/second. Ninty visible contractions were observed at the gastrocnemius muscle every day for 21 days. In the control group the electrodes were attached the same way as the experiment group but the device was closed.

At the 21st day the sciatic nerve was exposed surgically and electrophysiological studies were repeated as mentioned above. The rabbits were euthanized with overdose pentobarbital sodium.

Statistical analysis: Distal motor latency, DCMAP, PCMAP and NCV were analyzed with repeated measurement of variance analysis. The mean difference of intervals and comparison of groups were analyzed with Duncan's multiple rang test and Bonferroni test. All reported values represent the mean plus or minus the standard deviation of the mean. For all tests $p<0.05$ was accepted as significant.

RESULTS

The contribution of pulsed direct current on nerve regeneration after the crush injury was evaluated electrophysiologically. Sciatic nerve paralysis was observed in all rabbits at the second day after the crush injury. In all rabbits, the sciatic nerve could be stimulated with 1-3 mA doses from the beginning to the end of observation period.

Initial data recorded after the sciatic nerve exposed and just before crush injury were 1.26±0.14 ms, 38.57±16.13mv, 43.35±16.88mv, 74.18±29.38 m s^{-1} and the values recorded just after the crush injury were 1.21±0.12 ms, 36.28±14.35 mV,10.05±7.03 mV, 58.36±16.50 m s^{-1}, respectively as distal motor latency, dCMAP, pCMAP and NCV. The distal motor latency and dCMAP was not changed after crush injury. However, the NCV and pCMAP were changed significantly after crush injury.

The values recorded after treatment (21st day) for the experiment group were 1.29±0.09 ms, 21.38±11.72 mV, 21.23±10.53 mV, 59.16±20.60 m s^{-1} and for control group 1.22±0.18 ms, 17.36±7.05 mV, 19.79±8.41 mV, 49.76±7.16 m s^{-1} respectively as distal motor latency, dCMAP, pCMAP and NCV.

Distal motor nerve latency was not significantly different between groups and intervals (initial, just after injury and after treatment). The mean values for dCMAP have not changed with intervals for both experiment and control groups. In experiment group, mean dCMAP was not significantly different between three intervals ($p<0.05$). However in the control group, the differences between initial and just after injury mean dCMAP values were not significant but it was significantly lower in post-treatment values according to the experiment group ($p<0.05$).

The post crush values of pCMAP amplitudes were approximately four times lower than the initial values. Mean pCMAP was significantly different at all intervals ($p<0.05$) but there wasn't any significant difference between each group. Mean NCV was different between physiologic and post-crush values but there was not a significant difference between groups.

DISCUSSION

Atrophy is inevitable in the effector muscle after nerve injury. When reinnervation is provided, muscle atrophy will be reversed. If the muscle tissue is replaced by fibrosis before the regeneration is accomplished, functional recovery cannot be seen (Hudson, 1983; Mumenthaler and Schliack, 1991). In the light of this knowledge, electrical stimulation of the denervated muscle is widely used in daily practice to reverse denervation atrophy and decrease fibrotic changes with appropriate blood supply (Karen, 1993). The beneficial effects of electrical stimulation on denervated muscles, especially after crush injury have been reported, but its effect after the repair of a complete cut remains controversial (Pollock *et al*., 1951). In peripheral nerve injuries, regenerated axons pass 0.25 mm of scar tissue per day, however when growing axons enter the endoneurial tube, their speed can reach 1-8 mm per day (Tercis and Smith, 1990). In this study, the effect of electrical stimulation on crushed sciatic nerve of the rabbit model was investigated based on electrophysiological parameters. We observed spontaneous recovery in all rabbits in both groups at the 21st day by means of electrophysiological data. When the two groups were compared at 21st day, there was no significant difference, except for the increase in dCMAP amplitude in the experiment group.

Pockett and Gavin (1985), in their study found that 0.1 ms square pulse electrical stimulation applied with a constant voltage stimulator for 1 h just after crush injury caused faster regeneration in the crush injured rat sciatic nerve, corresponding to 5, 10, 15, 30 min of stimulation. They used toe-spreading reflex to monitor nerve regeneration. Although this stimulation did not affect the number of axons regenerating, it had an effect on the speed of regeneration (Pockett and Gavin, 1985). Mendonca *et al.* (2003), in their study evaluated the effect of direct current on the regeneration of sciatic nerve of rats, using a model of crush injury using both functional and morphometric data. They found that an active circuit delivering 1 μA constant continuous electric current caused an improvement in both functional parameters and muscle fiber density. Electrical stimulation not only induced regeneration but delayed axonal degeneration as well (Mendonca *et al*., 1985). In our study, we maintained bipolar stimulation of the sciatic nerve for the contraction of the gastrocinemus muscle to induce nerve regeneration of crush injured sciatic nerve. Lack of functional monitoring data might be another limitation of our study to determine the exact time course for the beginning of the functional recovery.

Compound muscle action potential amplitude is the vectoral sum of action potentials occurring as a result of the contraction of all muscle fibers after the stimulation of the nerve. As the muscle fibers become thicker and increase in number, the CMAP amplitude is expected to be greater (Kimura, 1989). When the initial and post-crush data was analyzed, the obvious decrease in the NCV and pCMAP amplitude supported the serious injury occurred by the crushing procedure which caused less nerve fibers to be stimulated. The results of our study showed that at the 21st day of the study, both groups improved by means of increase in NCV and CMAP amplitude with no superiority of the groups to each other except for higher dCMAP amplitudes in the experiment group. Denervated muscle fibers cannot be contracted with nerve impulses. Atrophic fibers, even if they are reinnervated, contribute the CMAP amplitude scarcely. Both the atrophy before reinnervation and the difference in the velocity of regenerated fibers are responsible for the decrease in CMAP amplitude (Kimura, 1989). When our data was interpreted considering this knowledge, we may suggest that the muscle fiber was more protected or prevented from atrophy with electrical stimulation.

CONCLUSION

In conclusion, depending on the electrophysiological data, our results did not support the enhancement of nerve regeneration by electrical stimulation of the muscle with bipolar technique, in sciatic nerve crush injury model of rabbit. However, the higher level of dCMAP in experiment group at the end of the study can be accepted as a positive effect of electrostimulation on nerve injury. We suggest that further researches should be planned corresponding to different groups treated with either electrical stimulation of the denervated muscle or the injured individual nerve in experimental studies.

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