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Possibility of reinnervation and prevention of distal target organ atrophy following side to side neurorrhaphy to the intact nerve after end to end repair of proximal transected peripheral nerves

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Abstract
The aim of the study is to investigate the possibility of reinnervation and prevention of muscle atrophy following side to side neurorrhaphy to the intact nerve after end to end repair of proximal transected peripheral nerves in order to prevent distal target organ atrophy. For this, four groups each containing five Sprague–Dawley female rats were used. In group I, no surgical procedure was performed as a control group. In group II, side to side distal neurorrhaphy performed to the tibial and peroneal nerves after end to end repair of transected proximal tibial nerve. In group III. distally side to side epineural neurorrhaphy performed to the tibial and peroneal nerves. In group IV, end to end epineural repair was performed after proximal tibial nerve transection. The rats were followed up for 3 months for nerve regeneration. Subsequently group II, III and IV were evaluated histopathologically. In all group, tibial and fibular bony weights, foot weights, anterior and posterior crural muscle weights and EMG parameters were evaluated. Comparison between the groups revealed no significant differences regarding EMG and muscle weights between groups 2 and 4 also axonal degeneration was observed in 3 group after neurorrhaphy. As result of experimental study, we think that side to side repair of intact distal nerves as an adjunct to end to end repair of proximal nerve transections has no additional benefit to prevent distal organ atrophy but rather may be caused harm on the intact nerve. In addition, it has been observed that this technique affects the intact nerve rather than the transected nerve.

Keywords: Proximal nerve transection, end to end, side to side, nerve repair

Introduction

Proper nerve repair is critical but not sufficient for complete rehabilitation of damaged and denervated tissues. As a result of the better understanding and combination of nerve injury and regeneration, significant improvements have been made in nerve repair. However peripheral nerve surgery has some problems. [1]. Among the factors affecting recovery in peripheral nerve transections, the level of injury is important. The more proximal the injury level, in other words, the closer to the medulla spinalis, injury causes more 2th motor neuron death. The decrease in the number of regenerated axons due to neuron death negatively affects functional recovery [2,3]. In the distal region, after the four weeks Schwann cells lose their regenerative ability due to loss of basal lamina and endoneurial tube fibrosis [4]. Another problem in proximal nerve injury is the long distance of the way that axons have to proceeding. The axons should succeed the target reinnervation before the muscle groups in the target region irreversibly go to atrophy. The longer the route, the longer the nerve recovery. After nerve injury, human muscle biopsies have been showed significant fibrosis at 3 months of denervation. Fibrosis progressively increases over time and severe fibrosis occurs in muscle tissue at 11 months [5]. While the distal end of a damaged nerve goes to degeneration, it can attract axons to itself by stimulating axonal sprouting in the intact nerve [6]. Studies have shown that the epineurium is an obstacle for axonal progression [7].

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In this study, the nerve, transected from the proximal region was repaired end-to-end. Afterwards distally, it performed side to side neurorrhaphy to an intact nerve. After distal neurorrhaphy that performed side to side to an intact nerve, it was investigated whether axonal sprouting in the intact nerve would progress to the transected nerve and prevent atrophy. In addition, we investigated whether this method is superior to end-to-end repair.

Material and Methods

After the approval of the ethics committee, this study was performed in the Animal Research Center Laboratory. A total of twenty adult, female Sprague-Dawley rats with a mean weight of 222.1 g (range 196 to 232 g) were used in the study. The study consisted of four groups with five rats each group. Group I was used as the control group without any surgical procedure. Anesthesia of 20 mg/kg ketamine hydrochloride (Ketalar®) and 10 mg/kg Xylazine hydrochloride (Rompun®) were applied intramuscularly and the left hip of each rat. Surgical procedures were performed using standard dorsal right gluteal approach after shaved and wiped with betadine solution. In group II, the sciatic nerve, tibial and peroneal nerves were explored after standard dorsal incision was made on the right lower extremities of the rats. The tibial nerve was transected as proximal as possible and repaired end-to-end with epineural method using 10/0 nylon suture, immediately. Afterwards distally, an epineural window was opened to the facing sides of both tibial and peroneal nerves with two micro forceps just above the tibial nerve branching area (Figure 1a). In the next step, side to side epineurial neurorrhaphy was performed with 10/0 nylon sutures on both nerves (Figure 1b). In group III, tibial and peroneal nerves were explored after standard dorsal incision was made on the right lower extremities of the rats. Distally an epineural window was opened to the facing sides of both tibial and peroneal nerves with two micro forceps just above the tibial nerve branching area. Afterwards, side to side epineurial neurorrhaphy was performed with 10/0 nylon sutures on both nerves (Figure 1c). In group IV, the sciatic and tibial nerves were explored after standard dorsal incision was made on the right lower extremities of the rats. The tibial nerve was transected as proximal as possible and repaired end-to-end with epineural method using 10/0 nylon suture immediately (Figure 1d). Finally, a dressing was applied to the wound and the animals were left to recover. Postoperatively, operated legs of rats were not splinted. All rats were housed in standard cages with food and water ad libitum under a natural day/night cycle. There were no deaths in any group, and no ulceration, skin atrophy and harm to each other were observed in the operated extremities of all rats. After a period of three months surgery, all animals were fixed on a 30 x 20 x 2 cm wooden plate (without anesthesia) from all extremities and the needle EMG was performed with Dantec Cantata® EMG instrument. On the sciatic nerve, a stimulating bipolar electrode was placed to proximal of the repair area. Bipolar needle electrodes (record) were inserted to distal crus anterior (peroneal nerve innervated) and crus posterior (tibial nerve innervated) muscles. In both muscle groups, presence of spontaneous activity (fibrillation) and MUP (Motor Unit Potential) properties were examined. Later, latency and amplitude of the muscles were measured with proximal stimulation. Following the EMG, all the animals were sacrificed with overdose of ketamine. Nerve tissue specimens were taken both from end-to-end repair sites and distal segments in group II and III. Sections of 5 microns in thickness were cut from the prepared paraffin blocks, stained with hematoxylin and eosin and then examined with a light microscope. In the all groups, at the right hind foot, anterior (peroneal nerve innervated) and posterior (tibial nerve innervated) muscles were detached from their insertion under microscope. These muscles and tibial bony, fibular bony, foot weights were calculated (sensitive scales precise to 10-3x g). The statistical analysis of the findings obtained in the study was performed using SPSS for Windows version 8.0 software. Comparison of the EMG findings between the groups was performed using analysis of variance. Pairwise comparisons were performed using the Mann-Whitney U test. Comparison of right hind foot muscles weight, tibia and fibula bony weight, foot weights were performed using one-way analysis of variance. A value of $p<0.05$ was accepted as statistically significant.

Results

When the peroneal and tibial nerve amplitudes were compared between the groups, the difference was not statistically significant ($p=0.178$, $p=0.71$). When the peroneal and tibial nerve latencies were compared between all groups, the difference was statistically significant ($p=0.027$, $p=0.022$). When the groups were compared as pairs in terms of tibial nerve latencies, distal latency prolonged in group II, III and IV compared to the control group ($p=0.008$). When the groups were compared as pairs in terms of peroneal nerve latencies, distal latency prolonged in group II and III compared to the control group ($p=0.008$, $p=0.0032$). The findings obtained from electrophysiological studies were as follows (Table 1). In group II, both peroneal and tibial nerve latencies were prolonged compared to the control group. There was degeneration in tibial and peroneal nerve and both nerves had been healed. In group III, tibial nerve latency was normal whereas peroneal nerve latency prolonged compared to the control group. There was degeneration both nerves and both of them had been healed. In group IV, tibial nerve latency was prolonged compared to the control group. There was degeneration in tibial nerve and had been healed. There was no statistically significant difference between posterior (tibial innervated) muscles, tibial fibular bony and foot weights. When the anterior (peroneal innervated) muscles weight were compared between the groups, the difference was significant ($p=0.000$) (Table 2). In Group II, there was axonal transport from the peroneal nerve to the tibial nerve at the side to side repair site. However, inflammatory granulation tissue was seen especially in the peroneal nerve. There was irregularity in the axon bundles of the peroneal nerve and minimal axonal degeneration. Suture granuloma was present both nerves (Figure 2). In the proximally, tibial nerve end-to-end repair site, there was regular axonal course and no inflammatory granulation tissue. In group III, axonal transport from peroneal nerve to tibial nerve was observed. Degeneration was present in both nerves and nerve sheath, axons had showed irregular distribution. Suture granuloma was present both nerves. In group IV. connective tissue was found less than other groups. Axonal passing in this group was more regular than the other groups and no signs of axonal degeneration were observed (Figure 3).

Table 1. Mean values of the results of electrophysiological evaluation of the groups

	Group I	Group II	Group III	Group IV
Tibial nerve Distal latency (m/s)	1.2±0.7	1.54±0.27	1.42±0.43	1.56±0.2
Peroneal nerve Distal latency (m/s)	1.04±0.5	1.51±0.25	1.52±0.584	1.34±0.48
Tibial nerve (M response) Amplitude (mv)	10.66±3.25	5.52±2.84	6.30±2.165	7.08±1.51
Peroneal nerve (M response) Amplitude (mv)	9.37±1.34	6.79±2.07	6.75±2.9	6.01±2.761

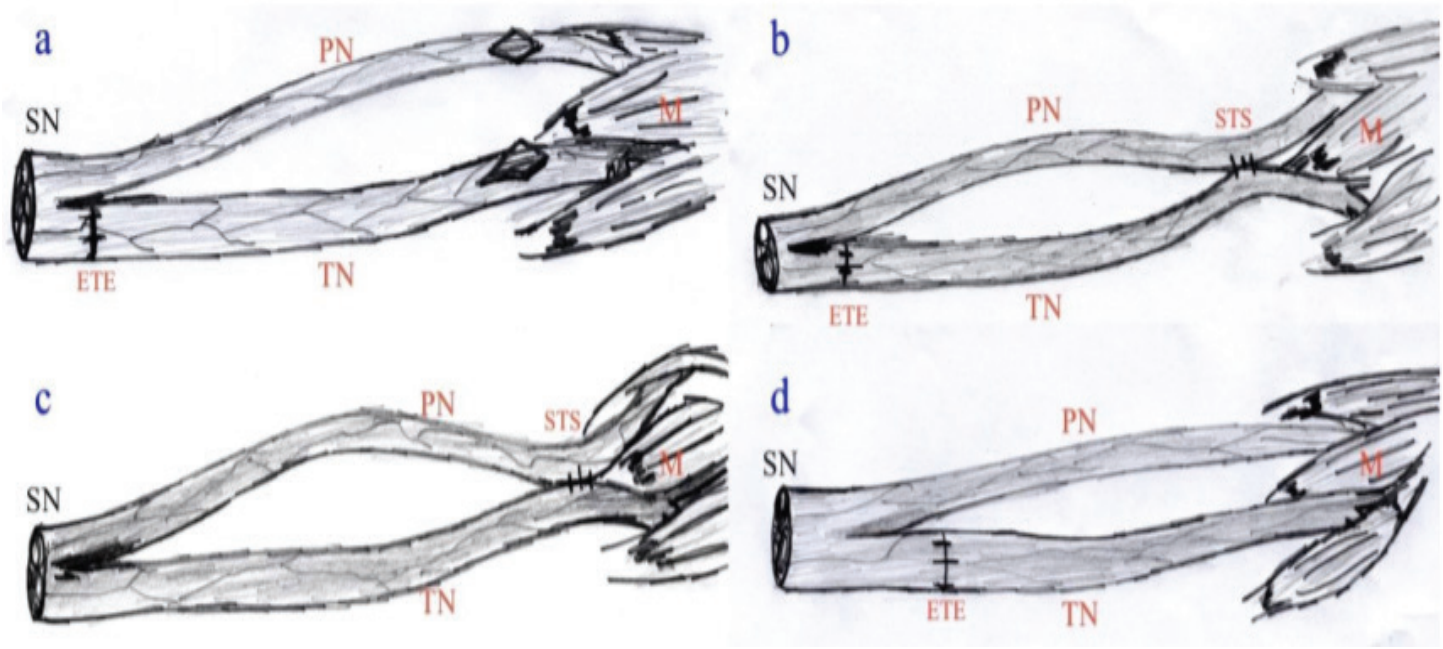

Figure 1. a, b group II, c group III, d group IV (SN Sciatic Nerve, TN Tibial Nerve, PN Peroneal Nerve, M Muscle, ETE End to End, STS Side to Side)

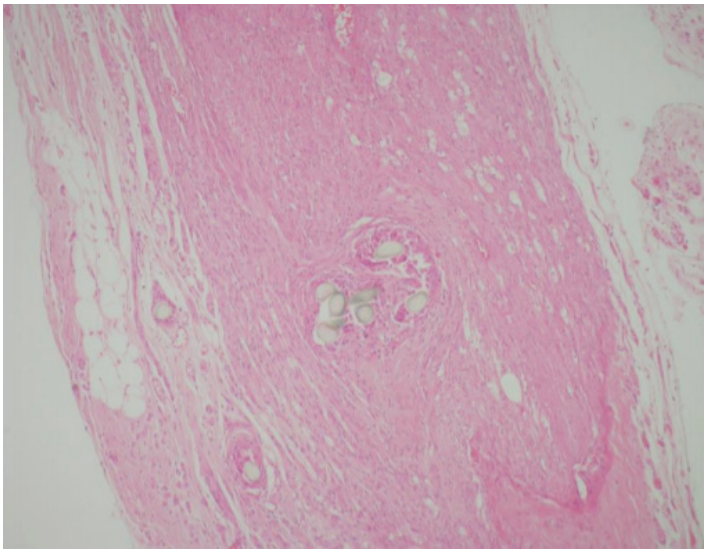

Figure 2. Axonal transport side to side neurorrhaphy site (X100, H-E)

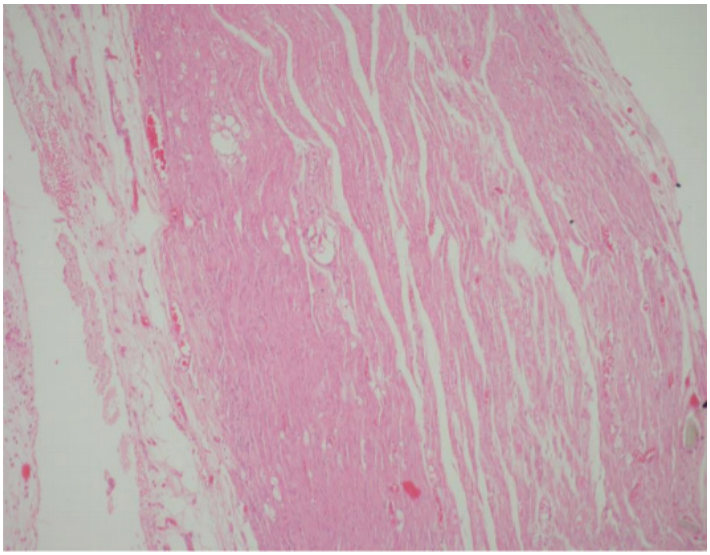

Figure 3. Regular axonal course in the tibial nerve end to end repair zone (X100, H-E)

Table 2. Mean anterior muscle and total body weight values of all groups

Group	Peroneal innervated (anterior) muscle weight (g)	Mean body weight (g)
I	0,5360 ± 0.0378	196
II	0.7140 ± 0.0451	232
III	0.8040 ± 0.01064	231
IV	0.6800 ± 0.0579	230

Discussion

In animal studies, denervated muscle tissue after peripheral nerve transection loses 50% to 60% of its total weight on day 60, with a 70% reduction in cross-sectional area. On the 89th day of denervation, fibroblastic proliferation does peak and collagen is stored. After 7 months of denervation, muscle can gain 87% of their previous contractile strength [8]. Fibrotic changes in muscles resulting from prolonged denervation limit functional recovery. These changes in the target organ becomes more important when a peripheral nerve is transected from a proximal region. In this case, even if the nerve is perfectly repaired end-to-end, the time taken for the axons to reach the target organ (distance of the injury to the cell body) will determine the final outcome. Retrograde transport of neurotrophic factors is interrupted after nerve injury.

This causes death of the motor and sensory neurons in the medulla spinalis and a lack of regeneration. Motor neuron loss increases as the injury approaches the medulla spinalis. [9]. In previous experimental studies, it was shown that the electrical stimulation given to rabbit muscles during denervation prevents muscle atrophy [10]. Embryogenic motor neurons were transplanted to the transected nerve, and this ectopic motor neuron pool has been shown to function as a functional electrical stimulus to maintain healthy muscle tissue during denervation. [11]. More interestingly, transplantation of sensory neurons into the nerve had the same effect [12]. Therefore, we thought to use an intact nerve in order to produce a similar effect during denervation in proximally transected nerve. Previous studies have shown that when the distal stump of transected nerve sutured to an intact nerve with end-to-side method, the collateral axonal sprouts formed in the intact nerve will pass to degenerate nerve [13]. This method is preferred when there is no proximal stump in nerve injury. Side-to-side nerve repair has been first presented by Yüksel et al. as an alternative to the end-to-side nerve repair technique [14].

In our study, the tibial nerve was transected as proximal as possible and repaired end-to-end with epineural method. Afterwards distally, an epineural window was opened to the facing sides of both tibial and peroneal nerves. In the next step, side to side epineurial neurorrhaphy was performed on both nerves (Group II). In order to evaluate the superiority of this repair method to end-to-end epineural repair method, group IV was formed. Group III was formed in order to monitor the changes occurring in the side-to-side nerve repair area and to observe the results. When the peroneal and tibial nerve amplitudes were compared between all groups, the difference was not statistically significant. But distal latency prolonged in group II, III and IV compared to the control group. The tibial nerve in groups II, III, IV and peroneal nerve distal latency were prolonged in groups II and III. It has been shown that when the epineural window is opened by pulling with the help of a pair of micro forceps, it does not cause any injury other than a few superficial axons just below the perineurium [7]. However, also there are studies that has been reported the opening the window in the epineurium of the intact nerve causes axonal damage and functional losses [13,15]. In our study, inflammatory granulation tissue was seen especially in the peroneal nerve in side to side repair area (group II). Also, there was irregularity in the axon bundles of the peroneal nerve and minimal axonal degeneration. In group III, degeneration was present in both nerves and nerve sheet, axons have been showed irregular distribution. Degeneration and alignment irregularity of the axons in the side-to-side neurorrhaphy area may have been due to the sutures that passing under the epineurium. However, in the end-to-end repair site axonal passing were more regular than the side to side neurorrhaphy and no signs of axonal degeneration were observed in group II and IV. There was no difference between posterior (tibial innervated) muscles, tibial fibular bony and foot weights. There was difference between groups in terms of anterior (peroneal innervated) muscles weight. Considering that no procedure was performed on the peroneal nerve in groups I and IV, we think that this was due to the difference in mean body weight between the groups (Table 2). When distal part of an injured nerve goes to degeneration, neurotrophic factors are released from non-neuronal cells in the distal segment [16]. However, contrary to the histological findings, anterior (peroneal innervated) muscle weights in group II and III being more than group I and IV may be due to this neurotrophic effect. Lacking of histopathological examination of the muscles whose innervation by the peroneal and tibial nerves are the weakness part of our study.

Conclusion

As a result of histopathological and electrophysiological studies, axonal degeneration of the intact and transected nerve was observed in the side to side nerve repair site. Nerve repair that performed with this technique did not contribute positively to the damaged nerve. In addition, it has been observed that this technique affects the intact nerve rather than the transected nerve.

Conflict of interest
The authors declare that there are no conflicts of interest.

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Ethical approval
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