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Effect of End-to-side Repair of Proximal Nerve Stumps of Transected Peripheral Nerves on the Development of Neuroma (Experimental Study)

Irfan Ayan,[1] Arslan Bora,[2] Mustafa Karakaplan,[3] Muharrem Inan,[4] Bora Bostan,[5] Burak Germen,[6] Suzan Zorludemir,[7] and Esin Atik[8]

[1]Department of Orthopaedics and Traumatology,School of Medicine, Mersin University, A.D. Zeytinlibahçe, Mersin, Akdeniz 33079 Turkey
[2]2nd Clinics of Orthopaedics, İzmir Atatürk Education and Research Hospital, Izmir, Türkiye
[3]Department of Orthopaedics and Traumatology, State Hospital, Malatya, Turkey
[4]Department of Orthopaedics and Traumatology, School of Medicine, Yeditepe University, Istanbul, Turkey
[5]Department of Orthopaedics and Traumatology, School of Medicine, Gaziosmanpasa University, Tokat, Turkey
[6]Department of Orthopaedics and Traumatology, Anadolu Private Hospital, Antalya, Turkey
[7]Department of Pathology, School of Medicine, Cukurova University, Adana, Turkey
[8]Department of Pathology, School of Medicine, Mustafa Kemal University, Hatay, Turkey
Irfan Ayan, Phone: +90-324-3374300, Fax: +90-324-3374305, Email: irfanayan@hotmail.com.
Corresponding author.
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Abstract

Objective
Neuroma is a psychologically and physically disabling problematic condition without any current standard therapy. For that reason, we investigated whether end-to-side anastomosis of the proximal end of the transected nerve into the adjacent nerve will prevent the development of neuroma in different types of nerve injuries.

Study design
In this study, hind legs of 18 Sprague–Dawley female rats were used. Six groups were formed. In group I, peroneal nerves were transected and its proximal end was attached end-to-side through the epineural window to the adjacent tibial nerve. In group II, contrary to group I, an epineural window was created in the tibial nerve and the same number of sutures were employed. In group III, tibial nerve was transected proximal to the end-to-side repair site, whereas in group IV, distal segment of the nerve was cut, and an end-to-end repair procedure was repeated. In group V, unlike group I, an approximately 1-cm segment was resected and removed distal (from tibial nerve) to the end-to-side repair site. In group VI, an epineural window was created in the tibial nerve and the same number of sutures were used, and also a 1-cm distal nerve segment was resected. The rats were followed for 2 months, and then all of the groups were evaluated histopathologically, and weights of the posterior muscle groups of hind legs were evaluated.

Findings and Conclusions
No neuroma formation was observed in the proximal stumps of peroneal nerve segments in end-to-side repair sites in groups I, III, IV, and V, and proximal stumps of the tibial nerve in group V. In group VI, neuroma formation was observed in the proximal end of the tibial nerve. When weights of the posterior muscle groups of hind legs in groups I and II were comparatively assessed, statistically significant difference was not detected. In conclusion, based on histological data obtained for proximal nerve ends and segments distal to the end-to-side repair sites, we think that end to side neurorrhaphy of the proximal end of the damaged nerve to adjacent nerve will prevent the development of neuroma without injuring the intact nerve segment.

Introduction

Neuromas that might develop at amputation stumps and after peripheral nerve injuries are currently challenging both to the patient and the physician with hard-to-treat clinical manifestations. Although various treatment modalities have been reported for the treatment of painful neuromas, definite and standard treatment modality has not been defined yet [2]. One of the relevant management alternatives is end-to-side neurorrhaphy of the proximal stump of the injured nerve to the adjacent nerve [2, 3, 15]. After demonstration of the development of reinnervation of distal target organs with end-to-side neurorrhaphy of distal nerve end to the adjacent intact nerve [5, 6, 9, 14, 16, 22], especially in cases with peripheral nerve injuries where proximal stump is not detected, this modality was suggested for the management of neuromas, and relevant studies have been conducted [2, 3, 15].
In our study, we investigated the impact of end-to-side repair of the proximal stump on the development of neuroma in different types of nerve transection injuries.

Materials and Methods

This study was performed in Laboratories of Animal Breeding and Research Center, after approval of the Ethics Committee. Twenty-four Sprague–Dawley rats (mean weight 248.8 g) with eight sciatic nerves were allocated to each group in our study. Unfortunately, six of the rats died (three from infection, two from cold weather, and one from anesthetic complication). For this reason, the study was performed in 36 sciatic nerves (peroneal and tibial nerve) of 18 rats. In groups I and II five, in group III and IV six, and in groups V and VI seven peroneal and tibial nerves were examined. One peroneal and tibial nerve of each group was examined under only electron microscope. Surgical procedures were performed under intramuscular Ketalar (ketamine hydrochloride 20 mg/kg) and Rompun (xylazine hydrochloride 10 mg/kg) anesthesia using standard dorsal gluteal approach (Fig. 1).

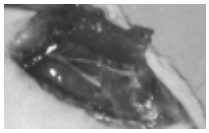
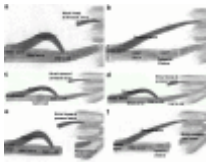


Figure 1
Intraoperative appearances of sciatic, tibial, and peroneal nerves.

Figure 1
Intraoperative appearances of sciatic, tibial, and peroneal nerves.
In group I, the peroneal nerve was cut, and its proximal end was repaired with end-to-side anastomosis performed through an epineural window onto the adjacent tibial nerve. In group II, to investigate the impact of end-to-side repair on donor nerve unlike group I, an epineural

window was created only in the tibial nerve using equal number of sutures. In groups III and IV, we tried to investigate whether the effect of distal or proximal localization of end-to-side repair site on the damaged tibial (donor) nerve would differ with respect to the formation of neuroma. In group III, in contrast to group I, the tibial nerve was transected proximal to the end-to-side repair site, and rerepaired. In group IV, the tibial nerve was transected distal to the end-to-side repair site, and rerepaired. In group V, an approximately 1-cm segment of tibial nerve distal to the end-to-side repair site was resected. In group VI, unlike group V, an epineural window into the tibial nerve was created with the same number of ligatures, and again a 1-cm segment was resected to imitate the amputation stump (Fig. 2a–f).

Figure 2



a Group I, **b** group II, **c** group III, **d** group IV, **e** group V, and **f** group VI.

Figure 2

a Group I, **b** group II, **c** group III, **d** group IV, **e** group V, and **f** group VI.
Both hind legs of rats were splinted for 1 week postoperatively, and followed up for 2 months for nerve regeneration. The rats were then killed with lethal doses of ketamine.
Nerve tissue specimens were taken both from end-to-side repair sites, and their distal segments for light and electron microscopic evaluation.
In groups I and II, posterior muscles (*musculus gastrocnemius* and *soleus*) were detached from their original insertion under microscope and than their wet weights were calculated with sensitive scales precise to $10^{-3} \times g$).
Tissues obtained were subjected to the procedures in the following order:

1. Nerve samples fixed with 10% formaldehyde were subjected to alcohol dehydration and xylol clearing and then blocked in paraffin. After serial cuts at five microtomes apart were performed with rotary microtome, specimens were stained with hematoxylin–eosin (H–E), Luxol fast blue–PAS, and Bielschowsky's reticulin stain and examined under light microscope (Nikon).
2. Tissue specimens fixed with 3% glutaraldehyde solution were left for 1 h in this solution. Afterward, these tissue samples, divided in one milimetric cuts were left in 3% glutaraldehyde solution for another 3 h. Tissue specimens were washed in the tampon solution overnight. Later they were refixed for 2 h in 1% osmium tetroxide prepared with Millonig phosphate tampon solution. Tissue specimens were serially dehydrated with gradually increasing concentrations of ethyl alcohol and embedded in epoxy resin.
    1. Cut sections at 1 μm thickness were stained with toluidine blue stain and examined under light microscope
    2. Thin cuts at 500 Ä thickness were stained with uranyl acetate and iron citrate, and examined under Zeiss EM 900 transmission electron microscope.

Data obtained were evaluated with nonparametric Mann–Whitney U tests using SPSS version 8.0 statistical software program.

Results

Muscle Weights

Table 1 shows that there is no significant difference between the weights of posterior muscle groups in groups I and II (p=1.000).

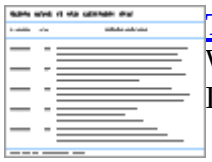


Table 1

Weights of posterior muscle groups and statistical evaluations in groups I and II.

Light and Electron Microscopic Findings

Groups I, III, IV, and V In sections obtained from end-to-side repair sites, the presence of proliferated axonal bundles, Schwann cells, and small myelinated axonal balls aside from suture granulomas were interpreted in favor of axonal regeneration. Axonal alignment was not disturbed apart from minimal degeneration seen distal to tibial nerve repair site (Figs. 3, ,4,4, ,5,5, and and66).

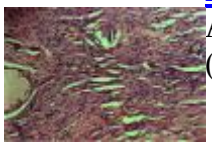
Figure 3

Axonal transport at end-to-side repair site (×400, H–E).

Figure 3

Axonal transport at end-to-side repair site (×400, H–E).

Figure 4

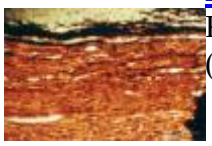


Axonal transport at end-to-side repair site. P = Peroneal nerve, T=tibial nerve (×400, H–E).

Figure 4

Axonal transport at end-to-side repair site. P = Peroneal nerve, T=tibial nerve (×400, H–E).

Figure 5

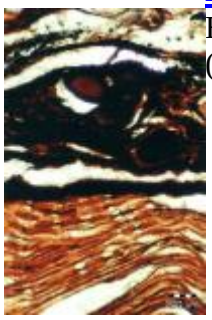


Regular axonal course in the tibial nerve distal to the end to side repair zone (×200, Bielschowsky’s reticulin stain).

Figure 5

Regular axonal course in the tibial nerve distal to the end to side repair zone (×200, Bielschowsky’s reticulin stain).

Figure 6



Epineural sutures at the and to side repair zone and regular axonal course (×400, Bielschowsky's reticulin stain).

Figure 6
Epineural sutures at the and to side repair zone and regular axonal course (×400, Bielschowsky's reticulin stain).

Group II As observed in cuts taken from the segment with epineural window, this procedure did not damage the intact nerve.

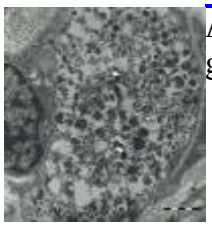
Group VI Unlike groups I, III, IV, and V, in group VI disorganized axonal proliferation pattern-like "neuroma" in amputation stump proximal to tibial nerve, irregular Schwann cell proliferation with surrounding connective tissue, and adipose and fibrotic tissue were encountered (Figs. 7, ,8,8, ,9,9, ,10,10, and and1111).

Figure 7

Appearance of neuroma (2×12,000, EM).

Figure 7
Appearance of neuroma (2×12,000, EM).

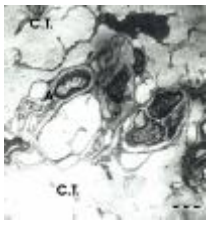
Figure 8



Appearance of neuroma at the cross-section from the proximal tibial nerve in group VI (×24,000, EM).

Figure 8
Appearance of neuroma at the cross-section from the proximal tibial nerve in group VI (×24,000, EM).

Figure 9



Appearance of neuroma (spars axons in proliferated connective tissue). C.T. =Connective Tissue, A=axon (×8,800, EM).

Figure 9

Appearance of neuroma (spars axons in proliferated connective tissue). C.T. =

Connective Tissue, A = axon (×8,800, EM).

Figure 10

Appearance of neuroma at the cross-section from the proximal tibial nerve in group VI (×200, toluidine blue).

Figure 10
Appearance of neuroma at the cross-section from the proximal tibial nerve in group VI (×200, toluidine blue).

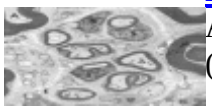
Figure 11

Appearance of neuroma at the cross-section from the proximal tibial nerve in group VI (×100, toluidine blue).

Figure 11
Appearance of neuroma at the cross-section from the proximal tibial nerve in group VI (×100, toluidine blue).

Electron Microscopic Findings In sections taken from end-to-side repair sites, axonal sprouting demonstrated in myelinated large axons, demyelinated axons, Schwann cells, and also minimal degenerative changes thought to be secondary to procedure performed on the repair site were observed. Any myofibroblast formation was not seen (Figs. 12, ,13,13, and and14).

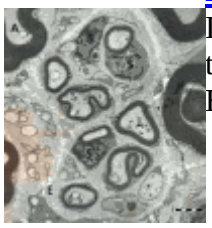
Figure 12



Axonal regeneration. S=Schwann cell, A=myelinated axon, E=endoneurium (2×4,400, EM).

Figure 12
Axonal regeneration. S=Schwann cell, A=myelinated axon, E=endoneurium (2×4,400, EM).

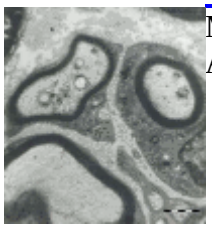
Figure 13



Intact axonal appearance at the cross-section in the proximal peroneal nerve at the end to side repair zone. S=Schwann cell, A=myelinated axon, E=endoneurium) (×8,800, EM).

Figure 13
Intact axonal appearance at the cross-section in the proximal peroneal nerve at the end to side repair zone. S=Schwann cell, A=myelinated axon, E=endoneurium) (×8,800, EM).

Figure 14



Myelinated axons at the cross-section from end to side repair zone. Asterisk=Endoneurium (×24,000, EM).

Figure 14
Myelinated axons at the cross-section from end to side repair zone. Asterisk=Endoneurium (×24,000, EM).

Discussion

In our study, end-to-side repair was seen to prevent the development of neuroma at proximal nerve endings without damaging the nerve repaired.

Fascicular structure becomes disorganized as a result of damage incurred with transection of the proximal end of the cut nerve. Deterioration in perineurium after transection leads to disintegration of fascicles swollen with edema, and decrease in their opportunity to coapt for regeneration. A rapid degeneration within one or two internodal intervals occurs after proximal axonal injury. This site prevents coaptation of regenerated proximal end with distal end of axonal sprout. As a result of this hindrance, axonal sprout shifts, branches, and neuroma develops [3, 10, 13]. For the treatment of neuroma, nearly 150 methods have been developed up to now [17]. Among these methods only resection; resection and anastomosing epineurium into the nerve stump; embedding in muscle, bone, and subcutaneous adipose tissue, placing inside a vein; coagulation or freezing; intraneuromal injection of chemical agents, placing a silicon cap on nerve ending; and centro-central (end-to-end) repairs can be stated [2-4, 10, 13, 15]. The objective of all these modalities is to prevent the formation of disorganized free nerve endings (fascicles) in sites blamed for painful stimuli and to also ensure proper alignment of regenerated axons [17].

End-to-side repair was performed in our study was thought to be used in peripheral nerve injuries where proximal nerve ending could not be found during surgical exploration. For the treatment of these type of injuries, various clinical and experimental studies performed have shown that end-to-side neurorrhaphy of damaged nerve to adjacent coursing intact nerve could be performed, and this modality could allow for reinnervation of distal target organs without damaging intact nerve [5–7, 14, 16, 20, 21].

After nerve injuries, axonal regeneration starts at proximal end proximal to the nodes of Ranvier; and for this regeneration, target organ and tissue specificity is not a prerequisite [3, 15]. In other words, proximal nerve endings absolutely regenerate even if essential (original) pathway and target are absent. In fact Low et al. [15] and Aszmann et al. [3] reported that for

the management of neuroma with end-to-side neurorrhaphy of proximal nerve ending to the adjacent nerve and establishment of a new target organ or pathway for proximal nerve ending will result in progression of regenerated axons at proximal nerve endings to these new targets, preventing the formation of neuromas. Al-Qattan [2] also conducted similar studies.
During histological evaluations in our study, neuromatous manifestations were only encountered at proximal endings of tibial nerve in group VI. Apart from these findings we did not detect neuromatous signs on proximal peroneal nerve endings within end-to-side repair regions, which were thought to be potential sites for neuroma development, and also at proximal tibial nerve endings in group V. Our findings, which are in compliance with those of previous studies mentioned in literature [2, 3, 10, 13, 15], reinforce evidences related to effectiveness of end-to-side neurorrhaphy of proximal nerve stump to the adjacent nerve in the management of neuroma, thanks to axonal transport achieved within this region.
Fairly successful outcomes have been reported both by Gorkisch et al. [8] who sutured proximal endings of fascicles inside a nerve using a central end-to-end technique, and Belcher and Pandya [4] who used either a graft between two nerve endings or direct centro-central (end-to-end) repair techniques. All of these studies as a prerequisite do not substantiate absolute presence of the primary target for axonal regeneration at proximal nerve endings. We attribute the healing without neuroma formation at proximal terminal of tibial nerve in group V to the resemblance of our end-to-side repair to an end-to-end repair method (because of closer vicinity) performed in some studies [2, 4, 13] with resultant orientation of proximal nerve endings to new targets and coaptation of proximal ends. In our study, lack of neuroma on end-to-side repair sites, and proximal terminals of peroneal nerve in groups III and IV, suggested that localization of end-to-side repair site proximal or distal to the tibial (donor) nerve transection line did not bear any significance with respect to the development of neuroma. In our study, any neuroma formation was not detected at proximal end of the peroneal nerve in groups III and IV. The reason for this was suggested to be attributed to Wallerian degeneration (distal to the transection) resulting from transections and repairs in tibial (donor) nerve, which might stimulate axonal transport at end-to-side repair site because of enhanced growth and regeneration accelerating factors in the vicinity. In fact, in the literature it is reported that degenerated nerve segments secrete nerve regeneration, growth, and neurotrophic factors (i.e., NGF, CNTF, and bFGF) [6, 7, 12, 19, 23].
Our histologic examinations revealed minimal axonal damage on segments distal to end-to-side repair sites of tibial nerve, nonexistence of Wallerian degeneration, and lack of difference in weights of posterior muscle groups between groups I and II, which reinforced the fact that end-to-side repair did not damage intact nerve in accordance with the literature findings (Table 1) [1, 6, 7, 11, 13, 18, 20].
In conclusion, end-to-side repair of proximal stump of a peripheral nerve to the adjacent nerve prevents formation of neuroma without injuring the donor nerve.

Acknowledgements

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