Effect of Nerve-Cutting Technique on Nerve Microstructure and Neuroma Formation

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Abstract

Background Peripheral neuroma formation results from partial or complete nerve division. Elucidating measures to prevent the development of peripheral neuromas is of clinical importance. The aim of this study was to determine the effect of various surgical nerve-cutting techniques on nerve microstructure and resultant neuroma formation.

Methods Twenty Sprague-Dawley rats were randomly assigned to one of the following nerve-cutting techniques: No. 15 scalpel blade with tongue depressor, micro-serrated scissors, nerve-cutting guide forceps with straight razor, and bipolar cauterization. The right sciatic nerve was transected using the assigned nerve-cutting technique. Neuromas were harvested 6 weeks postoperatively, and samples were obtained for histologic analysis. The contralateral sciatic nerve was transected at euthanasia and analyzed with histology and with scanning electron microscopy in a subset of the rats.

Results Fifteen of the 20 rats survived the 6-week experiment. Scanning electron microscopy of the No. 15 scalpel blade group showed the most visual damage and disorganization whereas the nerve-cutting guide forceps and micro-serrated scissors groups resulted in a smooth transected surface. Bipolar cauterization appeared to enclose the fascicular architecture within a sealed epineurium. Each neuroma was significantly larger than contralateral controls. There were no significant differences in neuroma caliber between nerve transection groups. No substantial differences in microstructure were evident between transection groups.

Conclusion Despite disparate microscopic appearances of the cut surfaces of nerves using various nerve-cutting techniques, we found no significant differences in the caliber or incidence of neuroma formation based on nerve-cutting technique. Nerve-cutting technique used when transecting peripheral nerves may have little bearing on the formation or size of resultant neuroma formation.

Keywords

- neuroma
- nerve microstructure
- nerve cutting
- nerve surgery
- peripheral neuroma

Introduction

Peripheral neuroma formation results from partial or complete nerve division. Following surgical transection, trauma, or extensive stretching, peripheral neuromas are formed as a result of abnormal axonal outgrowth originating from improperly regenerating proximal nerve stumps.^{1,2} The resulting neuroma is the biologic response of proximal and distal nerve ends failing to

rejoin, whereas a neuroma-in-continuity may form even after coaptation.³ Peripheral neuromas may cause chronic pain and altered sensation in the distribution of the peripheral nerve.^{3–5}

Approximately 20 to 30% of patients with peripheral neuromas are symptomatic with pain being the primary chief complaint.⁶ However, the treatment of peripheral symptomatic neuromas tends to be complicated and often results

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in poor outcomes.⁷ Though an abundance of therapeutic interventions exist for the management of symptomatic neuromas, a definitive treatment modality has yet to be established. Current treatment options range from rehabilitation and pharmacologic options to surgical intervention.⁸ Thus, elucidating measures to prevent the development of peripheral neuromas is of clinical importance.

Currently, a wide variety of nerve-cutting techniques are used in the operating room during procedures involving nerve repair, neuroma excision, and amputation.^{9–16} These range from sharp transection techniques (nerve-cutting guide forceps with straight razor, fresh scalpel blade) to techniques using various surgical scissors to nerve transection with cautery. Sharp transection techniques are thought to decrease symptomatic neuroma formation by uniformly limiting nerve fiber outgrowth whereas proponents of electrocautery believe that it decreases symptomatic neuroma formation by inducing a Sunderland fourth-degree injury, thus resulting in little to no functional recovery.^{4,14} However, the extent of injury induced by electrocautery is widely debated, and its role in reducing symptomatic neuroma formation is unclear.^{15–17}

To determine the effect of commonly used surgical nerve-cutting techniques on nerve micro-structure and subsequent neuroma formation, we used a Sprague-Dawley rat sciatic nerve transection model. The aims of this study were to (1) analyze the incidence and caliber of neuroma formation 6 weeks postoperatively and (2) compare the extent of damage to nerve structure and fascicular architecture through scanning electron microscopy (SEM) and histologic analysis. We hypothesized that neuromas would form at each proximal nerve stump despite the nerve-cutting technique used. Additionally, we hypothesized that nerve-cutting techniques using sharp, crush-free transection of nerve fibers (nerve-cutting guide forceps with straight razor) would result in smaller, less cellular neuromas than nerves transected with other methods (bipolar electrocautery, scalpel with tongue blade, micro-serrated scissors).

Methods

IACUC Approval

This study was approved by our Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with the guidelines established by our institution's Division of Laboratory Animal Medicine (DLAM).

Animals and Study Design

Twenty adult female Sprague-Dawley retired breeder rats with an average weight of 319 g were acquired from Envigo (Dublin, Virginia, United States) and housed in polycarbonate cages in groups of two or three. They were given standard food and water *ad libitum* and placed on a 12-hour light/dark cycle.

The rats were randomly assigned to one of four experimental groups (five rats per group) subjected to different surgical nerve-cutting techniques. These groups included (1) No. 15 scalpel blade on a sterile tongue depressor, (2) micro-serrated scissors, (3) nerve-cutting guide forceps and straight razor (Accurate Surgical & Scientific Instruments Corporation, Westbury, New York, United States), and (4) bipolar cauterization.

Surgical Procedure

Three days prior to the surgical procedure, the animals were given preoperative acetaminophen-treated water (250 mg/kg) for acclimation purposes and continued for 6 days postoperatively. All surgical procedures were performed under general anesthesia induced with 5% isoflurane inhalation in a 1:1 mixture of air and 100% oxygen. Anesthesia was maintained with 2.5% isoflurane. The right hind limb was shaved, prepped, and draped in the standard sterile fashion. A 1-cm transverse incision was made on the lateral thigh, and the underlying gluteal muscles were split in line with their fibers, exposing the sciatic nerve. The sciatic nerve was carefully exposed and freed from surrounding tissue. The trunk of the right sciatic nerve was transected 5 mm proximal to the sural nerve using one of the aforementioned randomly assigned nerve-cutting techniques. Following complete nerve transection, the proximal and distal nerve ends were carefully separated to reduce the likelihood of coaptation. The surgical incision site was closed using sterile wound clips.

Tissue Harvest and Preparation

Six weeks postoperatively, each rat was euthanized using CO₂ overdose followed by bilateral thoracotomy. The area overlying the surgical site was prepared in a similar fashion to the initial surgery. The right sciatic nerve was carefully exposed and freed from the surrounding tissue after opening the original skin incision along the lateral aspect of the thigh. The nerve was transected 3 mm proximal to the formed neuroma, and the most proximal end of the harvested nerve was marked. The contralateral nerve was subsequently harvested after transection using the same nerve-cutting technique used on the original experimental limb.

The diameters of each neuroma and contralateral nerve were measured at their largest diameter using digital calipers with an accuracy of ± 0.02 mm immediately upon harvesting.

Harvested nerves were fixed in 10% neutral buffered formalin overnight and dehydrated in 70% ethanol. Specimens were embedded and subsequently cut into 5- μ m cross-sections. Sections were then stained with 1% toluidine blue (TB), 0.1% Luxol fast blue (LFB), or hematoxylin and eosin (H&E) by standard protocol, then mounted and cover-slipped for imaging.

Scanning Electron Microscopy

A subset of freshly transected contralateral nerves was fixed and visualized using a Zeiss Supra 25 Field Emission Scanning Electron Microscope with a resolution of 2-nm (Zeiss United States New York, United States). The Zeiss microscope was fully computer controlled using a motorized goniometer stage. One specimen per nerve-cutting technique was visualized with the SEM, and images were studied for qualitative differences in the cut nerve surface between nerve cutting techniques.

Histomorphometric and Statistical Analysis

ImageScope (Aperio Technologies, Inc., Vista, California, United States) imaging software was used to perform nerve histomorphometric analysis of all digital slides. Harvested neuroma and contralateral controls were subjectively analyzed, and histologically measured cross-sectional area

measurements and nuclei counts per high-power field (HPF) were compared using one-way analysis of variance (ANOVA). A p -value of 0.05 was considered statistically significant.

Results

Fifteen of the 20 rats survived the 6-week experiment. The five rats that did not survive the duration of the experiment developed postoperative wound infections secondary to self-mutilation of their right hind limb and were euthanized according to our IACUC protocol. Contralateral sciatic nerves were obtained from the euthanized rats to be used for visualization under SEM for qualitative analysis.

The incidence of neuroma formation did not differ among our nerve-cutting techniques. The presence of newly formed neuromas was visually observed at the distal end of the right sciatic nerve 6 weeks following surgical transection in all specimens regardless of nerve-cutting technique used (► **Fig. 1**). Each neuroma was significantly larger than its respective contralateral control, with bipolar cauterization resulting in the largest formed neuroma (► **Fig. 2**). However, ANOVA determined no difference in cross-sectional area between our four nerve-cutting techniques ($p > 0.05$).

Fig. 1 Well-formed neuroma 6 weeks after surgical transection for qualitative demonstration of peripheral neuroma size and shape. Each peripheral neuroma was found to be adhered to surrounding musculature and was carefully harvested for analysis. An appreciable increase in size compared with proximal nerve structure was seen with each neuroma regardless of nerve-cutting technique used.

Fig. 2 Graphical representation of neuroma and contralateral nerve average cross-sectional areas. Neuromas formed after sciatic nerve transection using No. 15 scalpel blade, nerve-cutting forceps with straight razor and bipolar cauterization are significantly larger than contralateral controls ($p < 0.05$). Neuromas formed after micro-serrated scissors reach a similar cross-sectional area as other nerve-cutting techniques but did not reach statistical significance most likely secondary to loss of rodents due to self-mutilation. No difference was found when comparing neuromas from each group using ANOVA ($p > 0.05$). Error bars represent standard deviation.

SEM of freshly transected contralateral sciatic distal nerve ends at magnifications of 200X and 500X revealed varying degrees of neural architectural disturbance. The cutting techniques using micro-serrated scissors (►Fig. 3A, A') and the nerve-cutting guide forceps with a straight razor (►Fig. 3B, B') resulted in a smooth transected surface with uniform axonal distortion. Extensive disorganization and a lack of axon uniformity were seen when a No. 15 scalpel blade was used

(►Fig. 3C, C'). However, unlike the sharp, crush-free approaches, bipolar cauterization appeared to enclose the fascicular architecture within a sealed epineurium (►Fig. 3D, D').

Subjective histologic analysis revealed increased cellularity and varied amounts of axonal disorganization in harvested neuromas. Freshly transected contralateral nerves and neuromas harvested 6 weeks postoperatively were prepared and stained with LFB, TB, or H&E (►Figs. 4, 5). LFB staining

Fig. 3 Scanning electron microscopy (SEM) of control nerves following surgical transection. SEM revealed subjective differences in appearance of the transected nerve ends at 200X (A–D) and 500X (A'–D'). (A, A') Micro-serrated scissors and (B, B') nerve-cutting forceps with straight razor resulted in a smooth transected surface with fairly uniform axonal distortion. (C, C') No. 15 scalpel blade shows apparent axonal disorganization due to counter-pressure of a firm, rigid surface (tongue depressor). (D, D') Bipolar cauterization resulted in an enclosed fascicular architecture within a sealed epineurium.

Fig. 4 Histologic representations of transected control nerve endings show apparent discrepancies when compared with neuromas. **(A')** Luxol fast blue (LFB) staining of contralateral nerves depicts individual myelinated axons of uniform diameter. **(B)** H&E staining and **(C)** toluidine blue staining of transected nerve endings of contralateral nerves.

Fig. 5 Histologic analysis of harvested neuromas shows no significant difference in total average nuclei count per high-power field (20X) among the techniques. Subjective histological analysis revealed increased cellularity and varied amounts of axonal disorganization in harvested neuromas when compared with freshly transected contralateral nerve endings. **(A)** LFB staining of neuroma with apparent myelinated axonal disorganization. **(B)** H&E staining and **(C)** toluidine blue staining of transected nerve ends shows increased cellularity.

of control nerves depicted individual myelinated axons of uniform diameter (**►Fig. 4A**) whereas stained neuromas revealed myelinated axonal disorganization (**►Fig. 5A**). H&E staining of contralateral nerves and neuromas (**►Fig. 4B, 5B**, respectively) as well as TB-stained contralateral nerves and neuromas (**►Fig. 4C, 5C**, respectively) showed increased cellularity. Using the LFB-stained samples, we determined the average number of nuclei per HPF at 20X. Neuromas formed following transection with each nerve-cutting technique resulted in a significant increase in a number of nuclei when compared with contralateral controls ($p < 0.05$) (**►Fig. 6**). However, the number of nuclei per HPF was not significantly different among the four nerve-cutting techniques ($p > 0.05$).

Discussion

Peripheral neuroma formation is an inevitable consequence of surgical transection of any peripheral nerve. The bulbous swelling formed at cut proximal nerve sites may be associated with neuropathic pain that is often difficult to manage. Effective treatment of symptomatic neuromas remains elusive, and research surrounding prevention of symptomatic neuroma is limited.⁶

Similar to other studies using a rodent neuroma model, a 6-week timeframe was used as a threshold for neuroma formation in our investigation.¹⁸ As we hypothesized, our approach resulted in well-formed neuromas at the proximal end of each transection site regardless of the nerve-cutting technique used. Not consistent with our hypothesis, no statistical difference was found between neuroma size

and nerve-cutting technique used. Though no statistical differences were found, bipolar cauterization resulted in the largest formed neuromas and the No. 15 scalpel blade resulted in the smallest. Statistical analysis concluded that the cross-sectional area of peripheral neuromas between groups was independent of the nerve-cutting technique used. The gross differences in cross-sectional area are likely attributable to varied amounts of connective tissue stroma related to inflammation and collagen deposition.¹⁹

It is plausible that nerve-cutting techniques that result in neuromas of decreased caliber and cross-sectional area may inherently minimize the incidence of symptomatic lesions. Though previous studies have described the effect of bipolar electrocauterization on peripheral nerves, we are unaware of reports of nerve damage secondary to other nerve-cutting techniques.^{13,20} Our study investigated damage to nerve micro-structure and neuroma formation resultant from various nerve-cutting techniques but did not evaluate the relationship to neuroma formation and symptomatic neuroma pain. Further investigation is required to elucidate the relationship of symptomatic neuromas and the choice of nerve-cutting technique.

SEM revealed subjective differences in appearance of the transected nerve ends. The micro-serrated scissors and nerve-cutting guide forceps with straight razor resulted in a smooth transected surface with fairly uniform axonal distortion (**►Fig. 3A, A'** and **4B, 4B'**, respectively). These findings are in contrast to what is seen after transection with a No. 15 scalpel blade (**►Fig. 3C, C'**). Although these three approaches grossly result in a cleanly transected nerve with sharply

Fig. 6 Graphical representation of neuroma and contralateral nerve total average nuclei counts per high power field (HPF) (20X). Neuromas formed after sciatic nerve transection using No. 15 scalpel blade, micro-serrated scissors, nerve-cutting guide with straight razor, and bipolar cauterization have significantly more nuclei than contralateral controls per HPF ($p < 0.05$). No statistical difference in the average number of nuclei per HPF was found between different nerve-cutting techniques using ANOVA ($p > 0.05$). Error bars represent standard deviation.

defined edges, the No. 15 scalpel blade requires counter-pressure of a firm, rigid surface (tongue depressor). This crushing is likely a contributing factor to the observed disorganization of neural micro-architecture. These findings are in line with other reports analyzing conventional scalpel neurectomy.^{7,10} Unlike the other nerve-cutting techniques, bipolar cauterization of the rat sciatic nerve resulted in an apparent enclosed fascicular architecture within a sealed epineurium (►Fig. 3D, D'). However, despite this finding on SEM, the caliber of neuroma formation in the bipolar electrocautery group was similar to neuromas seen with other nerve-cutting techniques. Use of bipolar cauterization in the prevention and correction of peripheral neuromas is limited in the literature. Moradzadeh et al characterized the injury induced by bipolar electrocautery as a Sunderland third-degree injury in a similar rodent model at 3 and 6 weeks postoperatively, while also reporting minimal functional recovery.¹⁵ Our early findings correlate with their conclusions; however, future research is needed to understand how the mechanical effect of bipolar cautery acts on neural structural impairment and regeneration.

Subjective inspection of histologic samples showed increased cellularity and varied amounts of axonal disorganization in harvested neuromas when compared with contralateral controls. However, when objectively comparing the total average number of nuclei per HPF between nerve-cutting techniques using ANOVA, we determined that choice of nerve-cutting technique had no significant bearing on cellularity. It is plausible that cellularity is strictly dependent on the rate of axonal regeneration, which is not surprising, as disorganized microtubules, a large component of neuromas,

have been well documented to underlie the failure of axonal regeneration.²¹ Nonetheless, these data further support the notion that the nerve-cutting technique used has little effect on the subsequently formed neuroma.

There are several limitations to this study. First, the loss of rodents early in the study, due to postoperative infection secondary to self-mutilation, weakened the statistical power of the analysis. The self-mutilated rodents euthanized early were not equally distributed among analysis groups with three belonging to the micro-serrated scissors group ($n = 3$) and two belonging to the nerve-cutting forceps with straight razor group ($n = 2$). However, average cross-sectional area of each group's neuroma and contralateral control reached similar sizes regardless of the sample number. We believe that our results would remain unchanged if all the rodents were to have survived the duration of the study given the observed trends.

Another limitation to our study was the lack of objective histomorphometric outcome measures such as myelinated axon counts and their resultant cross-sectional area. Unlike other studies investigating peripheral neuroma formation in the rabbit forelimb, our approach did not include the use of quantifiable histomorphometric parameters to analyze histologic neuroma samples.²⁰ After consultation with neuropathologists at our institution, we found that the level of cellular disorganization present in the neuromas was great enough to make analysis of histomorphometric outcomes difficult to interpret for our samples, and thus we abandoned these outcomes.

Despite disparate appearances of the cut surfaces of nerves using various nerve-cutting techniques, we found no significant

differences in the caliber or incidence of neuroma formation based on nerve-cutting technique in a well-established neuroma model. We conclude that the nerve-cutting technique used when transecting peripheral nerves may have little bearing on the incidence or size of resultant neuroma formation. Further animal and clinical studies are necessary to determine the clinical applicability of these data.

Conclusion

The findings of this study suggest that though microscopic appearance of cut peripheral nerve ends may differ between nerve-cutting methods, the technique used when transecting peripheral nerves may have little bearing on the formation or size of resultant neuroma formation.

Note

An abstract of this paper was on for electronic poster display at the American Society of Surgery the Hand (ASSH) 2016 Annual Meeting in Austin, Texas from September 27, 2016 to October 1, 2016. A podium presentation of this paper was given at the North Carolina Orthopaedic Society (NCOA) Meeting in Pinehurst, North Carolina, on 10/8/2016.

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Conflict of Interest

None.

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