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Fascicular Ligation in the Prevention and Treatment of Painful Terminal Neuroma: An Experimental Study in the Rat

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The role of microfascicular double ligation and interligature coagulation in the prevention of painful neuroma was studied in an experimental model of section of the sciatic nerve in the rat. The experimental parameters used were the onset of autotomy and the weekly autotomy score. The autotomy of the denervated limb is a behavioral pattern that occurs in the experimental animal after the severing of peripheral nerves and has been related to anomalous electrical activity originating from the neuroma. Microfascicular ligation was performed immediately after section of the sciatic nerve or 10 days later. The results were compared to those of control groups treated with simple nerve section or simple neuroma resection 10 days after nerve section. The results show that immediate or delayed microfascicular ligation has no effect on pain as measured by the development of autotomy. (*Neurosurgery* 21:215–217, 1987)

Key words: Fascicular ligation, Pain, Neuroma, Sciatic nerve

INTRODUCTION

Fascicular double ligation and interligature coagulation was recently proposed by Battista and Cravioto for the treatment of pain due to terminal neuroma (2–4, 6). This surgical technique is based upon the assumption that the perineurium is the only effective barrier capable of containing regenerating axons. Microfascicular ligation has been successfully used on a small group of patients (2, 4, 6). Electrophysiological studies performed by Tamas and Howe on the saphenous nerve of rats treated with this surgical procedure (11), however, showed that microfascicular double ligature and interligature coagulation produced no changes in the conduction velocity of the nerve or in the abnormal spontaneous activity or mechanosensitivity originating in treated neuroma when compared to control neuroma. On the other hand, spontaneous abnormal activity from the neuroma bulb has been correlated by Wall et al. with the autotomy that follows the experimental section of peripheral nerves in the rat (13).

In an attempt to obtain further experimental information on the value of fascicular double ligation and interligature coagulation in the prevention of pain due to terminal neuroma, we performed this experimental animal study to investigate the role of microfascicular ligation on the time course of autotomy after experimental transection of the sciatic nerve in the rat.

MATERIALS AND METHODS

A total of 50 adult Sprague-Dawley male rats weighing 250 to 300 g were used. The animals were deeply anesthetized with ketamine. In 20 animals, the sciatic nerve and its branches were exposed microsurgically in the left thigh and cleanly severed (Group 1—control group). In 20 rats, before sciatic nerve section, a microfascicular double ligation and interligature coagulation was carried out as described by Battista and Cravioto (3). Initially, the tibial, sural, peroneal, and cutaneous branches of the sciatic nerve were dissected under magnification in the thigh (9). Each branch was isolated and then tied with a double ligature of 8-0 silk, leaving 5 mm between the knots. The surface of each branch between the ligatures was then electrocauterized, with care taken to avoid the production of blisters, scabs, or ruptures on the perineurium. Finally, each branch was severed distally (Group 2—immediate microfascicular ligation group). A 5- to 10-mm segment of each distal branch stump was resected in all cases to prevent spontaneous reinervation. Finally, in 10 rats the left sciatic nerve was cleanly severed as in the control group but, 10 days later, one of the following was performed: simple neuroma resection (Group 3—neuroma resection group) or microfascicular ligation as in Group 2 after the removal of the terminal neuroma (Group 4—delayed microfascicular ligation group). In all animals, the surgical wounds were closed by layers and no antibiotic, topical, or parenteral drugs were administered.

All animals used in the experiments were housed under identical colony conditions. The animals were individually housed. The observation period after the first surgical procedure was 10 weeks long. All animals were examined every 48 hours by a member of the investigational team who was unaware of the experimental group of each individual animal. The area of hypesthesia and the degree of autotomy were recorded. The experiment was conducted within the guidelines established by the International Association for the Study of Pain for research experiments in chronic animal models of pain (7).

The extent of autotomy was quantified in accordance with the scale proposed by Wall et al. (13): 1 point scored for the loss of one or more nails, a further point for each half digit attached, an additional point for autotomy of a metatarsal area, and another for autotomy of the tarsus. The maximal score possible was 13 points. The day of the first indication of autotomy (autotomy score ≥ 1) and the weekly autotomy score were used as experimental parameters. Statistical evaluation was then carried out using Student's *t*-test, the Mann-Whitney U test, and the χ^2 test with Yates' correction. A P value of less than 0.05 was considered significant.

At the end of the observation period, the proximal stump of the sciatic nerve was microsurgically exposed in all animals with the aim of evaluating its size and relationship to the surrounding tissues. The stump was removed from all animals for routine histological study, and the animals were killed.

RESULTS

There were no complications related to the surgical procedures. The injuries caused by heat shrinking were generally homogeneous and small in rats in which fascicular ligature

and coagulation were performed immediately after section of the sciatic nerve (Group 2). Four animals were dropped from the study because of excessive damage to the perineurium by heat. Denervated paws were paralyzed, and sensory response was lacking in the cutaneous areas corresponding to the sectioned nerves. In time, muscular atrophy developed; the area of anesthetized skin remained constant. The contralateral limb remained unaffected in all animals. None of the animals died during the observation period.

Autotomy

In Group 1, the mean time of onset of autotomy was 15.1 ± 12.3 (SE) days and, in Group 2 (immediate microfascicular ligation), it was 18.8 ± 13.3 days ($P > 0.05$). The mean times after section of sciatic nerve to the onset of autotomy were 14.4 ± 10.6 days in Group 3 (neuroma resection) and 15.6 ± 9.8 days in Group 4 (delayed microfascicular ligation) ($P > 0.05$). Some degree of autotomy was observed in all animals at the end of the experiment.

Table 1 shows the mean values of autotomy scores for each experimental group for each week after sciatic nerve section. The average score follows a similar course in all groups and autotomy is seen to increase with time, stabilizing in the final weeks. The time course of autotomy was not modified by immediate or delayed microfascicular ligation when compared with controls. There were no statistical differences among experimental groups, although the autotomy scores in Group 2 were slightly lower than those in Group 1.

Pathological findings

Microsurgical observation of the proximal stump of the sciatic nerves at sacrifice showed that the stumps of nerves treated with microfascicular ligation had smaller neuromas than the stumps in the control and neuroma resection groups. These neuromas were also free of adherence to the surrounding tissues except at their distal tips.

Light microscope study showed a typical terminal neuroma in animals in the control and neuroma resection groups, with axonal sprouts mixed with connective tissue and muscle. Specimens from the microfascicular ligation group showed conservation of the fascicular pattern. The interligature portion showed inter- and intrafascicular fibrosis. A large number of bundles of regenerative axons were seen beyond the most distal fascicular ligature, growing within vascularized connective tissue (Fig. 1).

DISCUSSION

Although autotomy has been considered as a simple response of the animal to an useless limb (10), there is evidence supporting the hypothesis that autotomy is a complex behavioral change undergone in the experimental animal because of a lesion of the peripheral nervous system (10, 12–14). The autotomy observed in rats after section of the sciatic nerve or brachial plexus has been related to anomalous impulses proceeding from the neuroma (5, 14). Moreover, this behavioral pattern has been modified by the use of drugs (14), neurostimulation (15), surgical lesions on the spinothalamic tracts (1), and surgical procedures involving the nerve stump, such as centrocentral anastomosis (8).

TABLE 1
Average Autotomy Scores of Control and Experimental Groups[a]

Week	Group 1	Group 2	Group 3	Group 4
1	0.9 ± 1.8	0.4 ± 0.5	0.5 ± 0.4	0.6 ± 0.5
2	1.7 ± 2.4	0.7 ± 0.6	1 ± 1.1	1.6 ± 0.8
3	2.1 ± 2.4	1.6 ± 2.9	1.8 ± 2.0	2.2 ± 1.3
4	3.5 ± 2.9	1.9 ± 2.6	2.8 ± 1.7	3 ± 2.5
5	4.7 ± 2.8	2.5 ± 2.8	3.5 ± 3.1	3.2 ± 2.6
6	5.1 ± 2.7	3 ± 3.2	3.5 ± 3.1	3.5 ± 3.0
7	5.2 ± 2.6	3.1 ± 3.2	4 ± 3.5	4.7 ± 3.8
8	5.4 ± 2.5	3.3 ± 3.5	4.2 ± 3.3	4.7 ± 3.8
9	5.7 ± 2.1	3.7 ± 3.1	4.8 ± 3.5	5.1 ± 3.5
10	5.5 ± 2.1	4.1 ± 2.9	4.8 ± 3.5	5.2 ± 3.7

[a] Values are means ± standard error. Group 1, nerve section; Group 2, immediate microfascicular ligation; Group 3, delayed neuroma resection; Group 4, delayed microfascicular ligation.

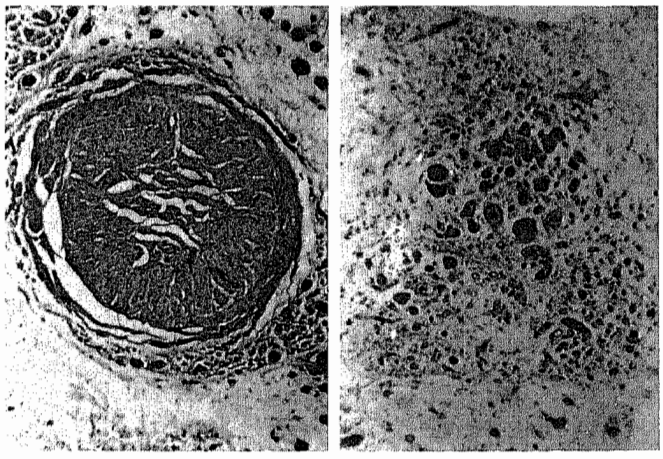

FIG. 1. Photomicrographs showing cross sections of a sciatic nerve with microfascicular double ligation and interligature coagulation (Group 2). *Left*: In the interligature portion, perineural and interneural fibrosis is visible. Some extrafascicular clusters of regenerating axons are seen around the nerve. *Right*: Distal to the second ligature, there are a large number of regenerative clusters growing out of the nerve fascicles. (Hematoxylin and eosin, × 100.)

The results of our experimental study show that autotomy is not altered by immediate or delayed microfascicular ligation when the animals included in these experimental groups are compared with those in control and neuroma resection groups, in which the neuroma is formed freely. Tamas and Howe did not find changes in electrophysiological parameters studied in the saphenous nerve of the rat when microfascicular ligation with interligature coagulation was compared to a simple nerve end ligature (11). Their report and the current study are at variance with the clinical results reported by Battista and Cravioto (2, 4, 6).

A possible explanation for our experimental results with fascicular ligation could be that, although such a technique exerts some change, it is unable to modify substantially the pathophysiological conditions produced in the painful terminal neuroma, which cause the electrophysiological and behavioral alterations in the rat. The aim of microfascicular ligation and cauterization is to enhance the role of the perineurium as a barrier to regenerating axon sprouts. Pathological studies show that these sprouts grow into endoneurial and perineurial scar in the interligature segment. Distal to the second ligature, however, groups of the regenerating axons are also seen invading the surrounding tissues (3). This probably reproduces some important factors related to the pathogenesis of pain caused by peripheral nerve lesions, namely the uncontrolled growth of regenerating axons within a scarred cellular and chemical environment (12). Moreover, several variables, such as tension of the knots, temperature of cauterization, thickness of the fascicles and perineurium, and possible injuries of the perineurium during microsurgical fascicular dissection make the final result uncertain.

Another factor to be considered in the model is the noci-

ceptive sensorial input during the operation at the moment of ligature and cauterization of each fascicle (12). It is obvious that the results of animal experiments on pain cannot be automatically extrapolated to the clinical field, although it is generally accepted that mammals possess an anatomical basis for pain similar to that of human beings and that animals can suffer pain.

In conclusion, we think that greater clinical experience and longer follow-up periods are necessary for the evaluation of microfascicular ligation and interligature coagulation, primarily because experimental results are consistent but contrary to reported clinical results.

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COMMENT

The authors use a fairly simple but practicable indicator of pain in a lower phylum animal to decide whether a relatively complex method of treating the severed nerve to prevent neuroma is effective. Since autotomy rates were equal in treated and untreated groups, fascicular ligation with intervening coagulation does not seem, at least in the rat, to be effective against neuroma pain. Using a different approach, Tamas and Howe studied the physiological effects of such a treatment regimen on rat neuromas and did not find evidence to support, again in the rat, the efficacy of this procedure. As pointed out in prior discussions concerning the original papers about this technique as well as Tamas and Howe's paper, the final evaluation must await carefully done long term trials of human nerve section and human nerve neuroma. One must keep in mind that, historically, the more complex the treatment of the severed nerve, the less effective the regimen seems to be (at least in the human setting) in preventing symptomatic neuroma formation. For the last decade, I have preferred placing the sharply sectioned nerve in as good a bed of surrounding soft tissue as possible and utilizing the bipolar and the microbipolar forceps under magnification to coagulate the tips of each of the exposed fascicular bundles. I have neither experimental data nor enough long term follow-up to prove the efficacy of this procedure and thus can only point out what I am currently doing.

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