

Nerve Grafts with Double End to Side Anastomosis: Results of Single and Double Grafts in Rats

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ABSTRACT

End to side nerve anastomosis is becoming an important tool for nerve repair. The use of a nerve graft with double end to side anastomosis was shown to be effective experimentally. Double grafts were however not studied. In the current study, a comparison between single and double grafts with end to side nerve anastomosis was conducted on 45 rats. Electrophysiological and morphometric evaluation of the results were done 16 weeks after the anastomosis. The results showed no statistically-significant difference between the single and the double grafts in the rat model. However, the data confirmed the possibility of the regenerating axons to cross an intact epineurial window.

INTRODUCTION

To borrow axons from an intact nerve for the sake of enervation an injured one is a very appealing idea. It has been used by surgeons for over a hundred years. At the beginning of the twentieth century Balance et al. [1] sutured the distal end of an injured facial nerve to the side of the spinal accessory nerve for treatment of facial paralysis and observed return of facial movements associated with shoulder movements. Kennedy suggested a similar technique but with more intense incision in the donor nerve and it also yielded good results [1]. Despite this, the technique was abandoned for more than 50 years until rediscovered by Viterbo et al., in the beginning of the 1990's by a series of experiments demonstrating growth of axons from an intact tibial nerve in the rat to the distal segment of a severed peroneal nerve. The experiments were done with and without removal of an epineurial windows and both resulted in collateral sprouting [2,3]. Viterbo further applied the technique on five facial paralysis patients with good results [4]. Lundborg et al., in 1994 supported the idea of collateral sprouting [5], while other authors claimed that morphologic proof by direct axon staining or electron microscopic evaluation were missing and the technique needs further assessment [6].

During the following 15 years different authors studied the end to side technique, some comparing different degrees of injury to the donor nerve and their effect on regenerating fibers [6], others compared the effect of leaving the epineurium intact or removing an epineurial window [7,8]. The major bulk of studies done confirmed the efficiency of the end to side technique for peripheral nerve repair. The technique was also used by some surgeons in the clinical setting for treatment of facial nerve injuries as well as upper extremity nerve injuries [4,9]. The technique of a nerve graft placed in an end to side fashion was also studied and showed adequate axonal regeneration [10]. However the value of multiple nerve grafts, placed by a similar technique, was never investigated. The purpose of this study is to compare, the effects and the functional results, of one and of two grafts placed in an end to side fashion between a donor and a recipient nerve.

MATERIAL AND METHODS

The study was done on 45 Sprague Dawley male rats with an average weight of 200gm. The microsurgical animal work was done at the Experimental Department at the Campus of Botucatu (Sao Paulo State University, Sao Paulo, Brazil) in the period from April to November 2006. The evaluation of the results was analyzed at the Department of Plastic Surgery, Ain Shams University.

The rats were classified into three groups of 15 animals. They were anaesthetized using intraperitoneal sodium pentobarbital (50mg./kg.). The right hind limb was completely shaved and sterilized using antiseptic solution. The animals were immobilized in the prone position on a wooden board. All surgeries were done using Karl Kaps surgical microscope with 12.5X magnification. A

longitudinal incision was done in the skin along the axis of the right hind limb. The same was done in the superficial muscle layer up to the pelvis exposing the sciatic nerve with its trifurcation into sural, tibial and peroneal nerves.

In group I rats (control), the sural nerve was harvested from the foot till its origin from the sciatic nerve. Closure of the wound was then done in one layer using 4/0 silk suture. In group II, two cm. of the sural nerve was harvested and kept in a gauze wet with normal saline. The peroneal nerve was then transected about 6-7mm. from its origin. The proximal stump was embedded in the gluteal muscles and fixed by a single 8/0 nylon suture. The distal stump was embedded in nearby muscles of the hind limb and also fixed by a single 8/0 nylon suture. A 7mm. graft (from the harvested sural nerve) was then obtained and placed between the distal segment of the peroneal nerve and the tibial nerve. It was fixed to both nerves in an end to side fashion using three 10/0 nylon stures without removing an epineural window (Fig. 1). The wound was then closed in one layer using 4/0 silk sutures. The same operative procedure was done for rats of group III with the placement of two grafts, 7mm. each, between the tibial and the distal peroneal nerves. The distance between the two grafts was about 4-5mm (Fig. 2). The rats had free access to food and water during the whole postoperative period till scarification.

After 16 weeks from the operative procedure, the animals were subjected to electrophysiological studies. They were then sacrificed for the morphometric studies.

Electric stimulation of the sciatic nerve was done and a receiving electrode placed in the right tibial cranial muscle (which receives its motor supply from the peroneal nerve). An electrical device was used to provide electrical impulses of 5.1 V. and a duration of 0.1ms. Repeated stimulation was done until there was no further increase in the recorded amplitude. In groups II and III rats, the grafts were transected and the stimulation was repeated. The results of amplitude and latency were printed out for the statistical analysis.

After the completion of the electrophysiological tests, nerve sections were harvested for the morphometric study. In group I, the specimens were obtained from the peroneal and the tibial nerves. In group II and III specimens were harvested from the peroneal and the tibial nerves at a level below the most distal anastomosis. Digital images of the prepared specimens were obtained and analyzed

using a special software (Sigmascan Pro 5) for the following data: The nerve fiber count, the average axon and the average myelin areas.

The data of the electrophysiological tests and the morphometry was expressed as mean and standard deviation for each group. Analysis of variance (ANOVA) was used to evaluate differences between group means.

RESULTS

All animals survived until the time of sacrifice, 16 weeks after the operative procedure. No infection or ulceration were observed. On exploration of the experimental groups the site of anastomosis in group II was identified with good attachment between the donor and recipient nerves and absence of neuroma or dehiscence. There was marked fibrosis on exploring the group III animals which increased the difficulty of obtaining the desired specimens.

Electrophysiological results revealed a positive response in all the animals in the control (I) and the experimental groups (II and III). This response became absent in the experimental groups after cutting the grafts connecting the tibial and peroneal nerves. The average recorded amplitudes for groups I, II and III was 24.9, 9.2 and 9.4mV., while the average latency was 1.4, 2.5 and 2.2mSec. respectively (Table 1, Fig. 3). Statistical analysis showed high significance between group I and both groups II and III, but there was no significance between group II and III.

Morphometric data of the obtained specimens are summarized in Table (2). The fiber count (Fig 4), the average axon area and the myelin area of the peroneal nerve were higher in group I than groups II & III. The results were statistically significant between group I and groups II & III and there was no difference between group II and group III. Regarding the tibial nerve, the morphometric results showed no statistical differences between the three groups.

Table (1): Electrophysiology: Mean and standard deviation of the average amplitude and latency of the three groups.

	Mean Amplitude (mV.)	Mean Latency (mSec.)
Group I	24.9091	1.4489
Group II	9.2722	2.5501
Group III	9.4553	2.2875

Table (2): Morphometric analysis: The average fiber count, axon area and myelin area of the peroneal and tibial nerves. The values are in square micrometer.

	Fiber Count (peroneal)	Fiber Count (tibial)	Axon Area (peroneal)	Axon Area (tibial)	Myelin Area (peroneal)	Myelin Area (tibial)
Group I	624.8	1647.8	3.2318	3.3101	2.3549	2.613457
Group II	247.4	1363.2667	2.3273	3.0459	1.8863	2.3284
Group III	187.9286	1429.0714	2.1269	2.9139	1.8037	2.273553



Fig. (1): A single graft applied by double end to side anastomosis between the tibial and the distal cut peroneal nerves (group II).

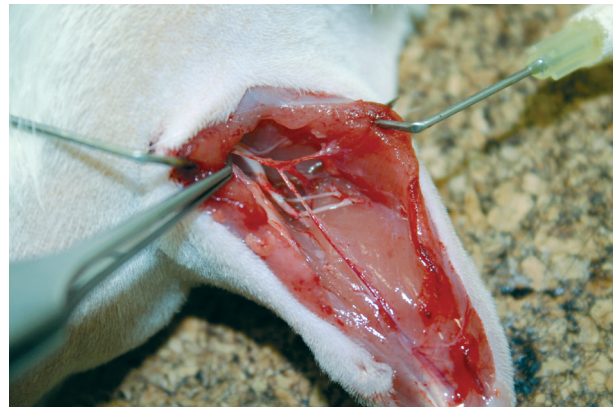


Fig. (2): Two grafts applied by double end to side anastomosis between the tibial and the distal cut peroneal nerves (group III).

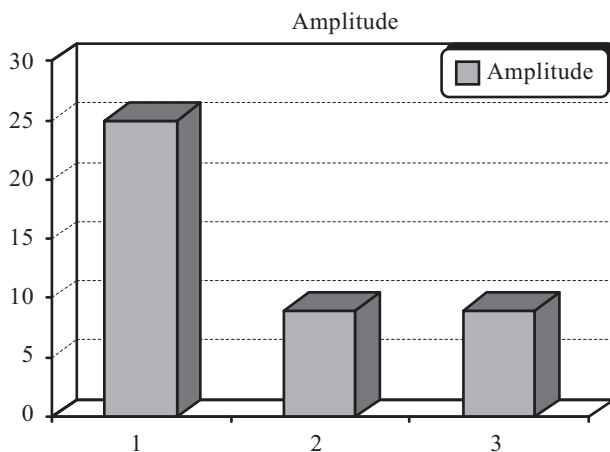


Fig. (3): Comparison between the average amplitude of the three groups.

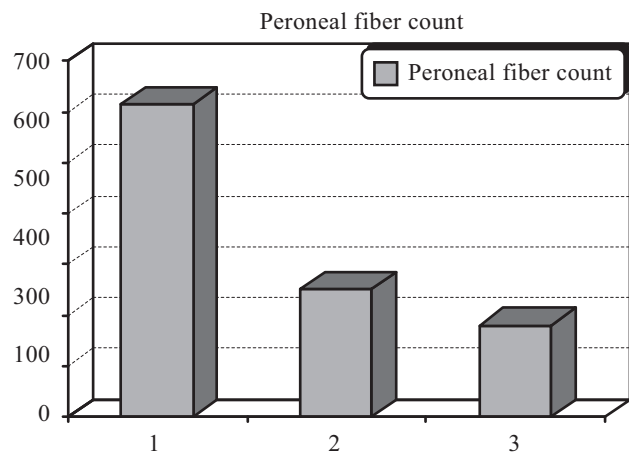


Fig. (4): The average fiber count of the distal peroneal nerve of the three groups.

DISCUSSION

End-to-side neurography has been extensively studied over the past 15 years, mainly experimentally and to a lesser degree in the clinical setting. Yet, a lot of controversial issues still exist. Regenerating axons, through the end to side anastomosis, was shown by electrophysiological, histological and morphometric analysis. The source of these axons, according to Rovak et al. [11], is either regenerating axons from the injured donor nerve, or true collateral sprouting from the injured recip-

ient nerve and the degenerating muscle fibers. A second common debatable issue is the ability of regenerating fibers to traverse the intact epineurium. According to Viterbo et al., the presence of epineurial sheath is not obstructing to the axonal growth through it with conductive properties [3]. Other authors emphasized the importance of removing an epineurial window in order to obtain a proper number of axons crossing the repair site. They even mentioned that the best results come with transecting a part of the donor nerve [5,6]. The results of the current study contradict the previous

conclusion pointing to the need to open an epineurial window. Electrophysiological results revealed responses in all animals of experimental groups although no epineurium was removed. This was further confirmed by the morphometric analysis. However, Zhang et al. [7], stated that axons crossing these layers in rats does not mean the same will happen in humans, as these layers are thinner in rats than in humans.

In the present experiment we were trying to increase the number of fibers regenerating across the end to side repair site with minimal or no injury to the donor nerve. A comparison of one and two grafts placed between donor and recipient nerves with double end to side anastomosis was conducted. In 1994, Viterbo et al. [10] reported efficient regeneration through a double end to side anastomosis with a graft placed between tibial and peroneal nerves in rats. The results of the fiber count in the distal peroneal nerve, in our study, was slightly lower than their results, but this might be explained by the difference in the scarification time of the rats. They sacrificed their rats after an average of 7.7 months instead of 4 months (of the current study).

Possible contamination from the proximal stump of the peroneal nerve was avoided in this study by placing the distal and proximal stumps in two different muscles. This proves that the axons detected in the distal segment of the peroneal nerve came from the tibial nerve and through the grafts.

In our results the axon count in the peroneal nerve in groups II and III were less than half that of group I, while there was slight difference between them. This was similar to the results of the electrophysiological studies as regards the amplitude and the latency. The axon and the myelin areas in group I were larger than the experimental groups (II & III). Between groups II and III there were only minor differences.

Although there were two grafts in group III, yet the results were not statistically different from group II (one graft). The addition of a graft was accompanied by more dissection which was evident during obtaining the nerve specimens. Animals in group III showed severe fibrosis and there was difficulty in harvesting the specimens. This fact might be due to the small size of the experimental animal and the results might be different if the same work is done in a higher animal model.

In the tibial nerve morphometric studies, all data were very close in all three groups with slightly

higher results in group I. The results were in accordance with those previously published [4,10,11], and indicate that the injury to the donor nerve is minimal.

Conclusion:

It is possible for axons from the donor tibial nerve to cross the intact epineurium and pass through the graft to the distal segment of the severed peroneal nerve. The presence of two grafts appears to offer no advantage over one graft. This fact might be due to the small size of the animal with very little space between the two grafts. We recommend performing this experimental model on a higher animal to obtain a more conclusive data.

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