

Peripheral Retrograde Nerve Regeneration After Sciatic Nerve Injury in a Rat Model: A New Concept in Peripheral Nerve Surgery

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Abstract

Background: Neuroregeneration after nerve injury occurs via anterograde (proximal in the distal) regrowth. The purpose of this study was to demonstrate that nerve regeneration occurs not only proximally to distally as classically described, but also distally to proximally using a rat model with a left sciatic nerve injury, with or without repair, compared to a healthy uninjured control group.

Methods: Fifteen rats were divided into 3 groups; a healthy control (Group 1, n=5), left sciatic nerve injury (Group 2, n=5), and left sciatic nerve injury and surgical retrograde nerve repair (Group 3, n=5). In Groups 2 and 3, complete surgical resection of the left sciatic nerve was performed and retrograde reconstruction of the nerve was done in Group 3 using an original, unique technique. Neuroconductive and histological variables were analyzed and compared between groups.

Results: After 15 weeks, Groups 1 (control) and 3 (retrograde reinnervation) behaved similarly, with no significant differences ($p < 0.05$) in the proportion of Schwann cells and axons, amplitude waves, nerve conduction velocity, and latency response time ($p < 0.05$), whereas Group 2 (injured unrepaired nerve) was significantly different from Groups 1 and 3 ($p < 0.05$). The reconstruction of injured sciatic nerves showed histopathological and neuroconductive characteristics similar to that of the healthy control group 15 weeks after the reinnervation process.

Conclusion: This preclinical study demonstrates that retrograde nerve regeneration after sciatic nerve injury is feasible in a rat model 15 weeks after reconstructive nerve surgery. These results indicate that neuroregeneration is possible in a caudo-cephalic direction.

Keywords: Peripheral nerve regeneration; Retrograde; Sciatic nerve injury

Received: September 26, 2016; **Accepted:** October 13, 2016; **Published:** October 18, 2016

Introduction

Neuroregeneration is a mechanism of repair and regrowth of nervous tissues, cells, or cell products after injury. The peripheral nervous system has an intrinsic ability for repair and regeneration. In contrast, repair of neurons in the central nervous system is very limited [1]. The capacity for regeneration is related to the age of the patient, mechanism of the injury, and the level of the injury (more distal injuries have better clinical outcomes) [2]. The pathophysiological mechanism of nerve regeneration remains unclear [3]. Damage to the spinal cord interrupts vital ascending

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Citation: Zermeño-Rivera JJ, López-Martínez CH, Fajardo-Barajas D, et al. Peripheral Retrograde Nerve Regeneration After Sciatic Nerve Injury in a Rat Model: A New Concept in Peripheral Nerve Surgery. J Univer Surg. 2016, 4:4.

and descending fiber tracts of spinally-projecting neurons. Because neuronal structures located proximal or distal to the injury site remain largely intact, a major goal of spinal cord injury research is to develop strategies to reestablish innervation lost as a consequence of injury [4].

The concept of retrograde peripheral reinnervation does not presently exist because so far the only known nerve growth is proximal to distal (anterograde peripheral reinnervation). The aim of this study was to demonstrate the possibility of retrograde peripheral nerve (caudal to cephalic) growth in a rat model with left sciatic nerve injury and retrograde repair reconnections *in vivo*, providing opportunities for new nerve transfers.

Methods

Fifteen specific-pathogen-free male Wistar rats (age 12 weeks; weight $240 \text{ g} \pm 10 \text{ g}$) were divided into 3 groups; a healthy control (Group 1, n=5), those with a left sciatic nerve injury (Group 2, n=5), and those with a sciatic nerve injury followed by surgical retrograde nerve repair (Group 3, n=5). The rats were housed in cages at $23^\circ\text{C} \pm 2^\circ\text{C}$, with good ventilation and natural day and night lighting, and free access to food and water. All rats were acclimated for one week before surgery. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by local authorities.

In Groups 2 and 3, complete surgical resection of the left sciatic nerve was performed. In Group 3, nerve reconstruction was done after the injury under a protocol of retrograde reinnervation outlet graft donor area. All surgeries were performed under anesthesia, and all efforts were made to minimize the pain and distress of the animals.

Retrograde surgical nerve repair technique protocol

We designed an original and unique technique for retrograde nerve reinnervation in a rat model as shown in (Figure 1).

The donor area (right leg) was opened under an asepsis and antisepsis protocol, and the sciatic nerve identified as well as its division into the tibial and peroneal nerve branches. The nerve was taken as a Y-graft 1 cm in length from its location proximal to the distal branch, leaving the right leg without a sciatic nerve. The incision was then closed with nylon 4-0. Subsequently, the graft and the area receiving (left leg) the graft was opened and the left sciatic nerve was dissected, making a full neurotmesis in the proximal third. The point of the proximal neurotomy of the left sciatic nerve was made in this site with the graft termino-terminal, as well as the supercharged termino-terminal with the femoral nerve, using 10-0 nylon. The two distal ends of the graft were anastomosed 1 cm below the section of the distal end of the division of the left sciatic nerve in the tibial and fibular nerves. One end was anastomosed termino-lateral to the tibial nerve, and the other end was anastomosed termino-terminal to the peroneal nerve. Leaving the sectioned segment of the proximal receptor of the tibial and peroneal nerves free to regenerate in a retrograde direction (study area) (Figure 2). All anastomoses were made with micro suture (Ethilon 10/0) prior to closing the incision.

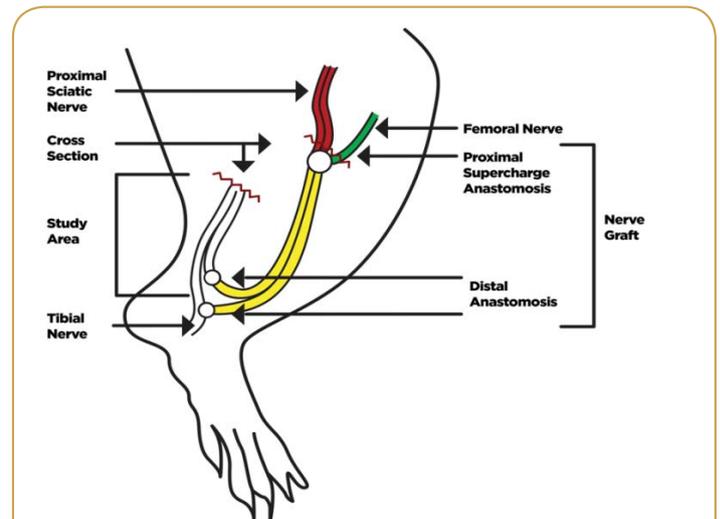


Figure 1 Description of the original and unique technique of retrograde nerve reinnervation in a left sciatic nerve injury in a rat model.



Figure 2 Application of the retrograde nerve repair technique in a complete lesion of the sciatic nerve in a rat.

After the operation, each rat was placed in its respective cage; later, the rats were moved to the care area where they were constantly monitored. They were fed and routinely cared for, taking special care of both hind legs.

Evaluation of nerve regeneration

Nerve conduction, histopathologic, and immunohistochemical studies of the sciatic nerves of the three groups were performed 15 weeks after the injury. Healthcare Vysis model Vicking quest was used to study nerve conduction. Histological and immunohistochemical tests (hematoxylin-eosin, indigo blue, Masson trichrome, and Cajal staining as well as antibody staining against CD20, CD68, CD34, specific enolases, and S-100) were used on tissue samples from the sciatic nerves.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 statistical software, Excel 2010. Non-parametric tests were

used and the differences between two continuous variables were determined using Kruskal Wallis test. Significance was established when p was less than 0.05, and confidence intervals were calculated at 95%.

Results

Fifteen rats were divided into 3 treatment groups and included in this study. **Table 1** shows the average values of the variables analyzed by treatment group. Significant differences were found between Groups 1 and 3 versus 2 for proportion of Schwann cells and axons, amplitude wave, nerve conduction velocity, and latency response time ($p < 0.05$).

Histological staining of the most representative nerves in each group is shown in **Figure 3**. Group 1 (**Figure 3A**) demonstrates the normal and healthy sciatic nerve structure, where normal cellularity is evident in all axons compared to Group 2 (**Figure 3B**), which clearly shows degenerated nerves with a loss of cellularity. Group 3 is represented in **Figure 3C**, showing a structure that is more similar to a normal nerve than the degenerated nerve in Group 2 (**Figure 3B**), with cellularity maintaining the nerve organization, epineurium, perineurium, and endoneurium.

Discussion

The surgical technique that was performed in this study to allow for retrograde nerve regeneration consisted of developing a neuroorrhaphy from a healthy donor nerve to a distal injured nerve segment, giving motor function to the proximal muscles in the area of the neuroorrhaphy for nervous growth in a distal-proximal direction.

On macroscopic histological examination, the sectioned sciatic nerve after surgical repair showed retrograde regeneration and reinnervation to adjacent muscle bundles (**Figure 4**).

In Group 1 with a healthy sciatic nerve, 94% Schwann cells, 1% fibroblasts, and other cells such as mast cells and capillaries were seen on the microscopic histological examination. On the reinnervated nerve, the histopathological analysis showed 91% Schwann cells, 2% fibroblasts, 1% mast cells, and 25% Bugner bands. Also observed was a thickened perineurium, an epineurium with abundant angiogenesis at the site of suture, and foreign body giant cells without granulomas, with an inflammatory infiltrate of lymphocytes. In the injured sciatic nerve, 5% Schwann cells were seen and the staining was negative for specific enolases. Additionally, almost 3% mast cells were

Table 1 Neuroconductive and histological variables analyzed in the three sciatic nerve groups.

	Group			P value ^a
	1	2	3	
Epineurium (thickness μm) $10\times$	32 ± 16.43	28 ± 13.03	56 ± 40.37	0.411
Perineurium (thickness μm) $10\times$	10 ± 6.12	5.4 ± 2.88	6 ± 2.23	0.306
Endoneurium blood vessels (thickness, $10\times$)	3.4 ± 1.67	3.2 ± 3.63	5.8 ± 3.70	0.443
Schwann cells (%) ($40\times$)	93 ± 2.73	11 ± 7.41	93 ± 2.73	0.001*
Axons (%) ($40\times$)	94.4 ± 0.89	3.6 ± 1.34	91 ± 2.23	0.000*
Mast cells (%)	1.2 ± 1.30	2.8 ± 1.92	1 ± 0.0	0.209
Fibroblasts (%)	1 ± 2.23	2 ± 4.47	2.2 ± 4.38	0.908
Wave amplitude (μV)	1732.4 ± 1918.38	0 ± 0	355.4 ± 337.33	0.000*
Nerve conduction velocity (ms)	30.8 ± 8.28	0 ± 0	13.4 ± 4.39	0.000*
Latency response time (ms)	0.88 ± 0.23	0 ± 0	1.66 ± 0.63	0.000*

^aKruskal Wallis test.

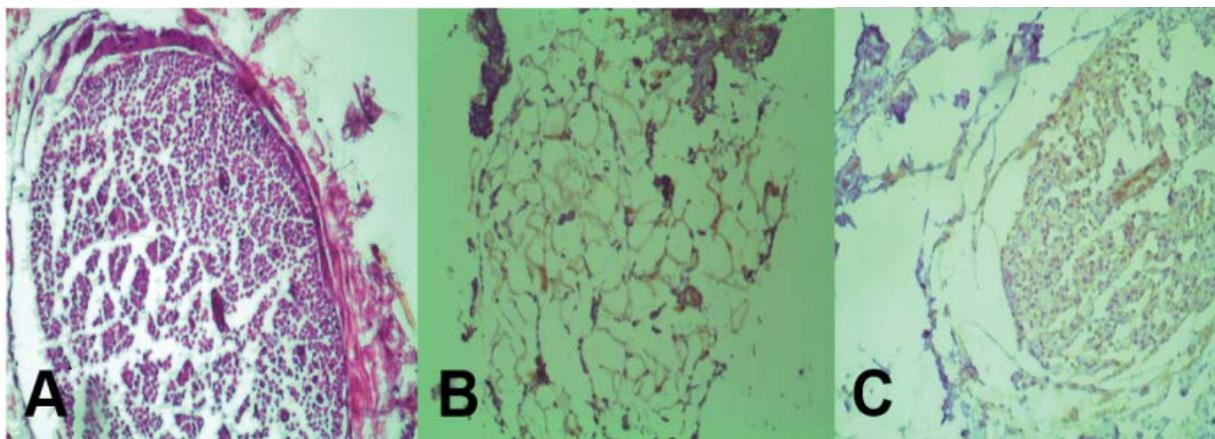


Figure 3 Photomicrographs of stained sections showing the most representative nerves in each treatment group. A: Group 1 – normal nerve with hematoxylin eosin staining, showing the epineurium, perineurium, endoneurium, and normal cellularity of axons. B: Group 2 – degenerated nerve with S-100 staining, showing evidence of loss of cellularity in the endoneurium, and no Schwann cells or axons. C: Group 3 – retrograde nerve reinnervation with S-100 staining, showing evidence of the epineurium, perineurium, and endoneurium. Axons and Schwann cells are marked, and there are 15% Bugner bands.

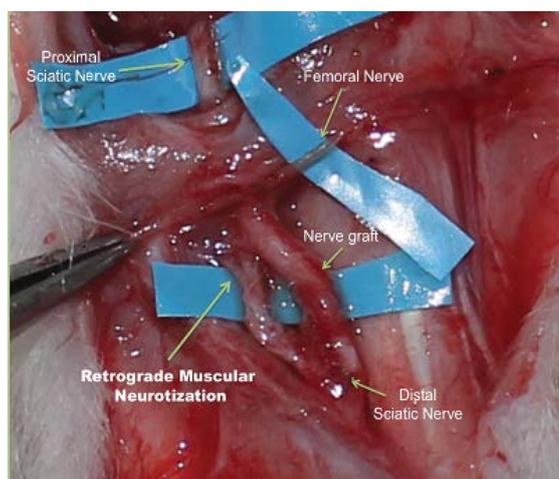


Figure 4 Neurotization zone of the dissected sciatic nerve in a rat.

found, which are involved in the inflammatory process, and angiogenesis was higher in the regenerated nerve compared to healthy nerves, although the difference was not significant, which is explained by the inflammatory process. All inflammatory processes initiate more blood vessels to repair the damaged tissue. By identifying the different dyes in reinnervated nervous tissue, the histopathological evidence in this study shows no significant intraneural inflammatory pattern when 10/0 sutures and the described technique (epi-perineural) were used. No histological changes were identified that could correspond to an acute inflammatory response. Cellularity corresponding to a chronic inflammatory response without forming granulomas was observed. Two or three Giant cells were located outside the endoneurium, which were enough to surround a 10/0 monofilament. Inside the endoneurium, macrophages and a few fibroblasts, identified by CD68 and CD34 staining, were found.

The neurostimulation study showed that the action potentials were similar in the reinnervated and healthy groups, but not in the degenerated nerves of Group 2.

The surgical technique used in this study for the reconstruction of the injured sciatic nerves showed histopathological and neuroconductive data that was similar to healthy nerves after

15 weeks of reinnervation. The study demonstrates that nerve regeneration in the caudo-cephalic direction may be possible, which has not been described and/or shown before.

One of the possible clinical applications of this new concept of "retrograde nerve regeneration" is to use new donor nerves, which are available from remote areas where the requirement for very long grafts limits reconstruction; openness to new nerve reconstruction techniques based on this principle, which allows for reinnervation in a caudo-cephalic direction; and to reach a proximal neuromuscular junction to innervate the same peripheral nerve in a distal direction.

It is also important to highlight that is the first study attempting to prove the theory of retrograde nerve regeneration, which has never been described in the literature. The limitations of this study are the small population size, evaluation of the variables over a short recovery time, and the absence of clinical evaluations. Additional studies are needed to confirm or refute these findings.

Conclusion

The principle of reinnervation in nerve reconstruction has so far been cephalocaudal. However, our study demonstrates neuroconductive recovery and histopathological evidence that retrograde peripheral reinnervation is possible in a rat experimental model, and the feasibility of rebuilding nerves in a caudo-cephalic direction.

Retrograde nerve reconstruction enables reinnervation to proximal muscles, generating a new concept of "retrograde reinnervation". This technique provides a new option for surgical nerve reconstruction in peripheral nerves. It is a feasible option in nerve reconstruction for those cases where it is not possible to use proximal nerve reconstruction.

Acknowledgments

The authors would like to acknowledge Luis E Figuera of the Division of Genetics, Biomedical Research Center of the West, IMSS, Guadalajara, Mexico, for his contribution to the general supervision of the research group. This study received no financial support.

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