HISTOLOGIC EVALUATION OF END-TO-SIDE NEURORRHAPHY. EXPERIMENTAL STUDY IN RATS

FABIANO INÁCIO DE SOUZA[1], GUSTAVO BISPO DOS SANTOS[1], CIRO FERREIRA DA SILVA[2], RAMES MATTAR JÚNIOR[1], ARNALDO VALDIR ZUMIOTTI[1]

ABSTRACT

Objective: The aim of this study was to histologically compare the axonal sprouting after end-to-side neurorrhaphy with or without epineurotomy. Methods: twenty male Wistar rats were used, divided into two groups of 10 rats each. A 1.0cm segment of the tibial nerve E was dried and sutured on the opposite side, where it was sutured into the sciatic nerve D. In Group I, the suture was made directly in the epineurium and in Group II, epineurotomy was performed. After 4 weeks, histological evaluation was carried out of the transposed segment and the sciatic nerve distal to the suture. Results: the results showed a small number of remyelinated fibers, varying from 7 to 51 fibers in Group I and from 10 to 91 fibers in Group II. The Mann-Whitney U test was used, with p=0.311, showing there is no statistically significant difference between the two groups. There was no positive relation between the number of remyelinated fibers in the graft and in the suture site distal to the sciatic lesion. Conclusion: lateral-ending neurorrhaphy, with or without epineural window, does not promote efficient remyelinization. Level of Evidence: Level II, prospective comparative study.

Keywords: Sciatic nerve. Nerve regeneration. Rats, Wistar.

Citation: *Souza FI, Silva CF, Mattar Júnior R, Zumiotti AV. Histologic evaluation of the end-to-side neurorrhaphy. experimental. Study in rats. Acta Ortop Bras. [online]. 2011;19(3):129-31. Available from URL:http://www.scielo.br/aob.*

INTRODUCTION

Peripheral nerve lesions are common and treatment methods are not yet able to foster fully satisfactory results, which awakens the interest of the scientific community. Among the techniques for microsurgical repair of injured nerves, end-to-side neurorrhaphy, which consists of the coaptation of an injured nerve segment, on the lateral surface of another adjacent intact nerve,[1,2] is the most controversial in medical literature.

The first reports on end-to-side neurorrhaphy date back to the beginning of the 20th century, where Kennedy *et al*. (1901) apud Al-Qattan[3] and Ballance *et al*. (1903) apud Pienaar[4], respectively, suggest its effectiveness in the treatment of facial spasm and in facial nerve paralysis.

Krivolutskaia *et al*.[5] and Viterbo *et al*.[6] reintroduced this technique almost a century later, affirming that there is lateral axonal sprouting to the receptor nerve without causing injury to the donor nerve.

Since then, numerous articles have been published both by advocates and by critics of this technique, transforming end-to-side neurorrhaphy into one of the main controversies of microsurgery.[7,8] The aim of this study is to perform a histological evaluation of axonal myelination in end-to-side neurorrhaphy, with and without epineurectomy.

MATERIALS AND METHODS

The study subjects were 20 young male Wistar rats, split into 2 groups of 10 animals. We anesthetized the animals with pentobarbital, in the concentration of 45mg/kg, administered intraperitoneally in the lower third of the abdomen. We promoted trichotomy, antisepsis and arrangement in the prone position. We made the longitudinal incision on the posterior side of the left paw, with dissection by planes and exposure of the sciatic nerve and its terminal branches. (Figure 1) We resected a 1.0cm segment of the left tibial nerve. On the contralateral side, the right sciatic nerve was dissected proximally, with the suturing of the right tibial nerve segment on its lateral wall. (Figure 2)

We did not perform epineurectomy in Group I, while an epineural window was created in Group II, using four stitches to suture the graft, applied at 3, 6, 9 and 12 hours. We used Mononylon 10.0 suture threads (Ethicon-Ethilon®, BV 130-4) and a DF

All the authors declare that there is no potential conflict of interest referring to this article.

1- Institute of Orthopedics and Traumatology of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo
2- Institute of Biomedical Sciences of Universidade de São Paulo

Study conducted at LIM 41 – Musculoskeletal Medical Investigation Laboratory of the Orthopedics Department of Faculdade de Medicina da Universidade de São Paulo.
Mailing address: Rua Dr Ovídio Pires de Campos, 333, 8º andar. Cerqueira César, São Paulo-SP. Email: fabianoinacio@usp.br

Article received on 09/30/08 and approved on 02/09/09.

Vasconcelos® microscope (Mod.MC-M3107), with 21x zoom. The sutures were performed avoiding axonal lesion of the right sciatic nerve. After 4 weeks, we euthanized the animals through the intraperitoneal administration of pentobarbital (150mg/kg). We made 1μm cuts in the graft segments and immediately distal to the suture in the sciatic nerve (Figure 3), where we conducted the histological analysis by means of the total count of the number of myelinated fibers.

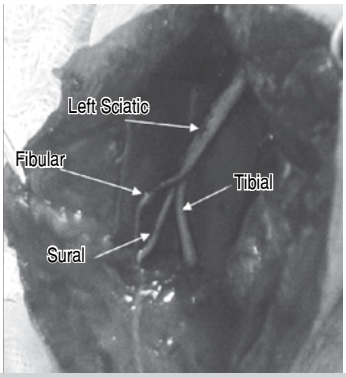

Figure 1. *Sciatic nerve and its branches.*

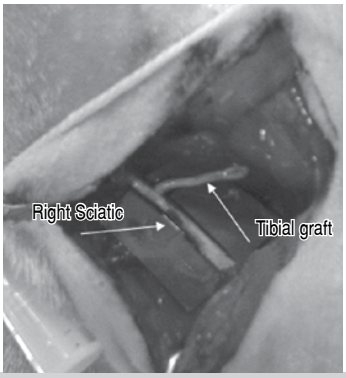

Figure 2. *Demonstration of the graft suture of tibial nerve E, to sciatic nerve D.*

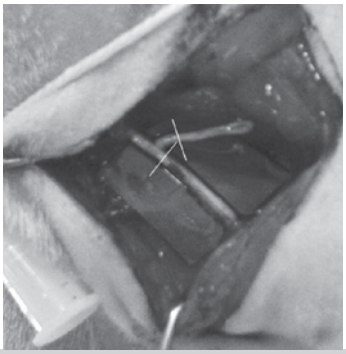

Figure 3. *Location of the histological sections.*

RESULTS

Groups I and II presented an extremely low number of remyelinated fibers. In Group I, the total number of remyelinated d axons varied from 7 to 51 fibers, while Group II presented variation from 10 to 91 fibers. We employed the Mann-Whitney U test, presenting p=0.311, demonstrating that there was no statistically significant difference between the two groups. (Table 1) We verified that there was no positive correlation between the number of myelinated fibers and the presence of sciatic nerve lesion focuses (caused during the suturing). (Figure 4)

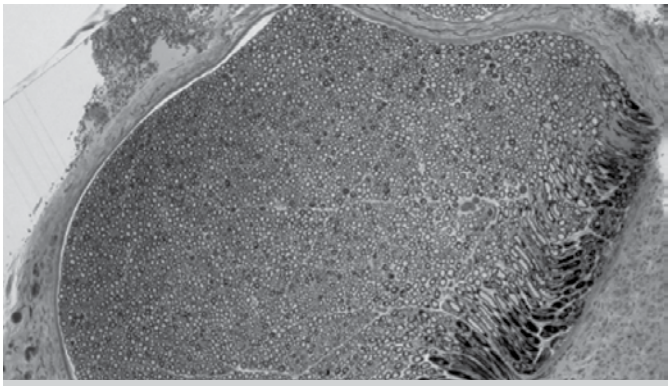

Figure 4. *Fixation of the medial epicondyle segment with a 3.5mm screw, with transfer of the flexor-pronator muscle group, 4cm proximal to the elbow joint.*

Table 1. *Mann-Whitney U test, one-tailed (p=0.311).*

	Group 1	Group 2
Mean	20	23.5
Standard error	4.546061	7.643806
Median	15	16
Mode	#N/D	12
Standard Deviation	14.37591	24.17184
Variance	206.6667	584.2778
Kurtosis	1.062643	8.989213
Asymmetry	1.274814	2.948604
Interval	44	81
Minimum	7	10
Maximum	51	91
Sum	200	235
Count	10	10
CVP	**71.87953**	**102.8589**

DISCUSSION

Nowadays, the best treatment after complete peripheral nerve injuries is direct coaptation of the stumps in the acute phase, using microsurgical techniques. In chronic lesions or those with segment loss, it is impossible to achieve stump coaptation without excessive tension. In these cases, nerve grafts are widely indicated. This technique does not produce full functional results, and causes injury to intact structures, condemning the patient to permanent sequelae, such as paresthesia, scars, risk of neuroma formation and chronic pain.[2,3]

This picture motivates researchers to seek treatment alternatives with better results. There are descriptions of several techniques, including tabulations through veins or the use of silicone tubes and end-to-side neurorrhaphy.[3,9]

Theoretically, end-to-side neurorrhaphy is said to promote, from a complete nerve, lateral sprouting of the axons reaching the target organ and restoring its sensitivity and motor function. It is a fast, simple and safe procedure, since it allegedly does not provoke lesions in the intact donor nerve, and would not injure another intact site.[6,10]

It would be an excellent strategy, if it were not surrounded by contradictions.[11] Viterbo *et al*. propose that lateral sprouting occurs irrespective of the performance of prior epineurectomy. Other authors believe that an epineural window would be essential for sprouting, as without this window it would not be possible for an intact nerve to emit a sufficient quantity of axons to transpose the epineurium and promote myelination of the "receptor" segment.[12,13]

Some researchers believe that the myelinated fibers contained in the receptor nerve result from the lateral sprouting secondary to the lesion provoked in the surgical procedure. This lesion apparently occurs during the performance of the epineurectomy or in the suturing process.[14]

Aiming to investigate this hypothesis, we prepared slides for histological evaluation at the site immediately distal to the coaptation of the receptor nerve. In some cases, the presence of axonal sprouting secondary to the small lesion area was demonstrated. However, there was no positive correlation between these lesions and a larger number of myelinated fibers. In the count, we verified that the total number of myelinated fibers, between 7 and 51 in group 1 and between 10 and 91 in group 2, is practically negligible when compared to the total number of fibers, 5133, of the normal tibial nerve of Wistar rats.[15]

While several articles demonstrate good results of end-to-side neurorrhaphy in humans,[10,16] others do not suggest its indication.[17,18]

For example, Fernandes *et al*.[9], after reviewing literature, affirmed the nonexistence of sufficient experimental or clinical evidence for the indication of end-to-side neurorrhaphy in humans. The same opinion is shared by Pienaar *et al*.[4], Pannicucci *et al*.[11] and Beris *et al*.[17]

Due to the extremely favorable and tempting concepts of end-to-side neurorrhaphy and of the significant difference of opinions in literature, the performance of new experimental studies with the addition of neurotrophic factors is imperative.

CONCLUSION

In the experiment performed, we concluded that end-to-side neurorrhaphy, with and without epineural window, does not promote efficient remyelination.

REFERENCES

1. Oğün TC, Ozdemir M, Senaran H, Ustün ME. End-to-side neurorrhaphy as a salvage procedure for irreparable nerve injuries. Technical note. J Neurosurg. 2003;99:180-5.
2. Ulkür E, Yüksel F, Açikel C, Okar I, Celiköz B. Comparison of functional results of nerve graft, vein graft, and vein filled with muscle graft in end-to-side neurorrhaphy. Microsurgery. 2003;23:40-8.
3. Al-Qattan MM. Terminolateral neurorrhaphy: review of experimental and clinical studies. J Reconstr Microsurg. 2001;17:99-108.
4. Pienaar C, Swan MC, De Jager W, Solomons M. Clinical experience with end-to-side nerve transfer. J Hand Surg Br. 2004;29:438-43.
5. Krivolutskaia EG, Chumasov EI, Matina VN, Mel'tsova GM, Kirillov AL. [End-to-side type of plastic repair of the facial nerve branches]. tomatologiia (Mosk). 1989;68:35-8.
6. Viterbo F, Trindade JC, Hoshino K, Mazzoni Neto A. Latero-terminal neurorrhaphy without removal of the epineural sheath. Experimental study in rats. Rev Paul Med. 1992;110:267-75. Erratum in: Rev Paul Med. 1993;111:479.
7. Papalia I, Geuna S, Tos PL, Boux E, Battiston B, Stagno D'Alcontres F. Morphologic and functional study of rat median nerve repair by terminolateral neurorrhaphy of the ulnar nerve. J Reconstr Microsurg. 2003;19:257-64.
8. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, Hunter DA. End-to-side neurorrhaphy resulting in limited sensory axonal regeneration in a rat model. Ann Otol Rhinol Laryngol. 1997;106:506-12.
9. Fernandez E, Lauretti L, Tufo T, D'Ercole M, Ciampini A, Doglietto F. End-to-side nerve neurorrhaphy: critical appraisal of experimental and clinical data. Acta Neurochir Suppl. 2007;100:77-84.
10. Mennen U. End-to-side nerve suture in clinical practice. Hand Surg. 2003;8:33-42.
11. Pannucci C, Myckatyn TM, Mackinnon SE, Hayashi A. End-to-side nerve repair: review of the literature. Restor Neurol Neurosci. 2007;25:45-63.
12. McCallister WV, Tang P, Trumble TE. Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J Reconstr Microsurg. 1999;15:597-603; discussion 603-4.
13. Rovak JM, Cederna PS, Kuzon WM Jr. Terminolateral neurorrhaphy: a review of the literature. J Reconstr Microsurg. 2001;17:615-24.
14. Zhang F, Fischer KA. End-to-side neurorrhaphy. Microsurgery. 2002;22:122-7.
15. Suad CA. Anatomia microscópica do plexo lombar e sacral de ratos Wistar. Tese de Neurologia. Faculdade de Medicina de Ribeirão Preto. Universidade de São Paulo; 2004.
16. Yüksel F, Peker F, Celiköz B. Two applications of end-to-side nerve neurorrhaphy in severe upper-extremity nerve injuries. Microsurgery. 2004;24:363-8.
17. Bertelli JA, Ghizoni MF. Nerve repair by end-to-side coaptation or fascicular transfer: a clinical study. J Reconstr Microsurg. 2003;19:313-8.
18. Beris A, Lykissas M, Korompilias A, Mitsionis G. End-to-side nerve repair in peripheral nerve injury. J Neurotrauma. 2007;24:909-16