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Correlation between functional, electrophysiological and histomorphometric parameters after rat sciatic nerve repair

Corelația între parametrii funcționali, electrofiziologici și histomorfometrici după sutura nervului sciatic de șobolan

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Abstract

Background. The high frequency of traumas during sports competitions led us to initiate an experimental study on peripheral nerve regeneration. Traumas mainly occur in the lower limbs, with an increased frequency in football, handball, rugby, basketball, skiing, bobsleigh. Peripheral nerve lesions have an incidence of 3% of all traumas and represent a significant clinical and social problem because patients frequently require several reconstruction operations, whose effects are often unsatisfactory. In certain cases, direct end-to-end neurorrhaphy is impossible post-traumatically, when there is a lack of nerve substance or when the proximal stump is inaccessible. A solution for the treatment of these cases is end-to-side neurorrhaphy.

Aims. The aim of the research is to determine whether end-to-side neurorrhaphy is a viable alternative to end-to-end neurorrhaphy and if some correlations can be established between functional, electrophysiological and histomorphometric data.

Methods. Functional, electrophysiological and histomorphometric data were obtained after transection of the rat sciatic nerve 1.2 cm proximal to its trifurcation and repair by end-to-side neurorrhaphy. At 16 weeks postoperatively, the sciatic functional index was determined and subsequently, the animals were re-anesthetized to determine nerve latency and the amplitude of the compound muscle action potential. According to the protocol, the animals were sacrificed for the histomorphometric study, which evaluated the number of fibers/mm², the mean diameter of fibers, the thickness of the myelin sheath and macroscopic nerve changes in the suture.

Results. Recovery after end-to-side neurorrhaphy is possible and can be explained by fiber regeneration from the lateral side of the donor nerve to the sectioned recipient nerve stump. There were no correlations between the sciatic functional index (SFI), electromyographic determinations and histomorphometric determinations, which were performed at the end of the follow-up period. A strong morphometric correlation was evidenced at the level of the distal segment between the number of fibers, their diameter and the thickness of the myelin sheath.

Conclusions. When the proximal nerve stump is absent, end-to-side neurorrhaphy is a real option, only when it is supported by an active nerve segment. There is no significant correlation between functional, electrophysiological and histomorphometric methods, which is seen in cases of large fascicle nerves; these methods show various aspects of nerve regeneration, and should be considered separately by therapeutic studies.

Key words: end-to-side neurorrhaphy, nerve regeneration, histomorphometric evaluation, sciatic functional index, injury in sport

Rezumat

Premize. Frecvenţa crescută a traumatismelor în cursul competiţiilor sportive ne-a condus spre un studiu experimental cu privire la regenerarea nervilor periferici. Traumatismele apar mai ales la membrele inferioare cu frecvenţă mărită în fotbal, handball, rugby, baschet, schi, bob. Leziunile nervilor periferici au o incidenţă de 3% din totalul traumatismelor. O soluţie în tratamentul cazurilor cu lipsă de substanţă nervoasă sau când bontul proximal este inaccesibil, o reprezintă sutura termino-laterală.

Obiective. Studiul urmăreşte dacă neurorafia termino-laterală a nervului sciatic de şobolan, după secţiune şi sutură poate fi o alternativă viabilă la sutura termino-terminală şi dacă există corelaţii între parametrii funcţionali, electrofiziologici şi histo-morfometrici.

Metode. În experiment s-au folosit 25 de şobolani. În toate cazurile, după expunerea nervului sciatic, s-a efectuat secţiunea acestuia la aproximativ 1,2 cm proximal de trifurcaţie şi apoi s-a efectuat repararea prin neurorafie termino-laterală cu fereastră epineurală. La 16 săptămâni postoperator s-a efectuat evaluarea regenerării nervului sciatic prin metode funcționale, utilizând

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indicele funcțional sciatic, electrofiziologice determinând timpul de latență și potențialul de acțiune muscular compus și histomorfometrice privind numărul de fibre/mm², diametrul mediu al fibrelor, grosimea tecii de mielină și modificările macroscopice ale nervului la nivelul suturii.

Rezultate. Recuperarea după neurorafia termino-laterală este posibilă și poate fi explicată prin regenerarea fibrelor de la nivelul feței laterale a nervului „donor" crescând spre bontul secționat al nervului „receptor". Nu există nici un fel de corelație între indicele funcțional sciatic (SFI), determinările electromiografice și determinările histomorfometrice, efectuate la sfârșitul perioadei de urmărire. Se evidențiază o corelație puternică în cadrul determinărilor morfometriei la nivelul segmentului distal între numărul de fibre, diametrul axonal și grosimea tecii de mielină.

Concluzii. În traumatismele nervilor periferici cu bont proximal nervos neviabil sau când sunt prezente defecte de nerv importante, neurorafia termino-laterală rămâne o variantă viabilă. Nu se evidențiază nici o corelație semnificativă între metodele de evaluare clinice, electrofiziologice și histomorfometrice, ele arată diferite aspecte a regenerării și ar trebui luate în considerare separat în studiile terapeutice.

Cuvinte cheie: sutură termino-laterală, regenerarea nervului, evaluare histomorfometrică, indicele functional sciatic, accidente în sport.

Introduction

The increased frequency of traumas during sports competitions and training, particularly in the lower limbs (57.70%) and in sports such as football, handball, rugby, basketball, skiing, bobsleigh, car racing (Untea, 2002), led us to an experimental study on peripheral nerve regeneration.

Nerve injuries depend on the mechanism and the force of trauma and can include individual nerve structure elements, which allow to categorize injuries depending on their severity. These occur in about 3% of all traumas and represent a major clinical and socio-economic problem, because patients require many successful or unsuccessful surgeries (Gligor, 2009). End-to-side neurorrhaphy is one of the alternative nerve repair methods when direct end-to-end reconstruction is not possible (De Medinaceli, 1995; Gligor & Georgescu, 2008).

Objectives

The study monitored the viability of regeneration of a distal nerve segment following an original end-to-side neurorrhaphy method, as well as quantitative structural and functional changes and the presence of correlations between functional, electrophysiological and histomorphometric evaluation methods.

Hypothesis

The research started from the hypothesis according to which transection of a peripheral nerve and suture of its distal end to an "active nerve" are followed by axonal sprouting, which occurs from the lateral side of the "active" nerve to the distal nerve stump sutured to it.

Material and methods

Research protocol

a) Period and place of the research

The research was conducted in white male Wistar rats, with a mean weight of 220±20 g, from the Biobase of "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca. The animals were kept under adequate standardized vivarium conditions, at the Center for Experimental Medicine and Practical Skills of "Iuliu Hațieganu" University of Medicine and Pharmacy. This experimental study was approved by the Ethics Committee of "Iuliu Hațieganu" University of Medicine and Pharmacy. The study was carried out in the period 2008-2010.

b) Subjects and groups

The experiment included 25 rats which were anesthetized intramuscularly with a ketamine and xylazine solution 0.13 ml/100 g (2/3 ketamine 5% and 1/3 xylazine 2%). In all cases, after the sciatic nerve was exposed, its transection 1.2 cm proximal to its trifurcation was performed. The rat sciatic nerve was repaired by end-to-side neurorrhaphy with an epineural window (Fig. 1). The sciatic nerve was transected and repaired with 10-0 prolene microsutures, under the operative microscope, by end-to-side neurorrhaphy using the following procedure: the distal end of the sectioned nerve was sutured to the lateral side of the proximal stump with an epineural window, 0.3 cm from the proximal end, which was previously ligated. Four suture points were used.

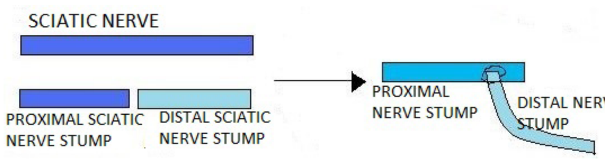


Fig. 1 – Schematic representation of sciatic nerve neurorrhaphy in the three groups.

c) Tests applied

Postoperatively, the rats were followed up for 16 weeks, during which the sciatic functional index (SFI) was determined at regular intervals: 2, 4, 6, 8, 10, 12, 14, 16 weeks, for clinical or functional evaluation. The parameters taken into calculation were the following:

- Print length *PL*: distance from the heel to the tip of the third toe.
- Toe spread *TS*: distance from the first to the fifth toe.
- Intermediate toe spread *ITS*: distance from the second to the fourth toe.

For the calculation of *SFI*, several factors are used, of which (Fig. 2):

- the print length factor: PLF=(EPL-NPL)/NPL;
- the toe spread factor: TSF=(ETS-NTS)/NTS;
- the intermediate toe spread factor: ITF=(EIT-NIT)/NIT

Where letter "E" represents the experimental part, and letter "N" the normal part.

The previously calculated factors were included in the

SFI formula, as follows:

SFI = -38.3*PLF + 109.5*TSF + 13.3*ITF – 8.83

Where:

SFI with the value “0” is considered normal;

SFI with a value of -100 indicates total involvement (e.g. complete transection of the sciatic nerve).

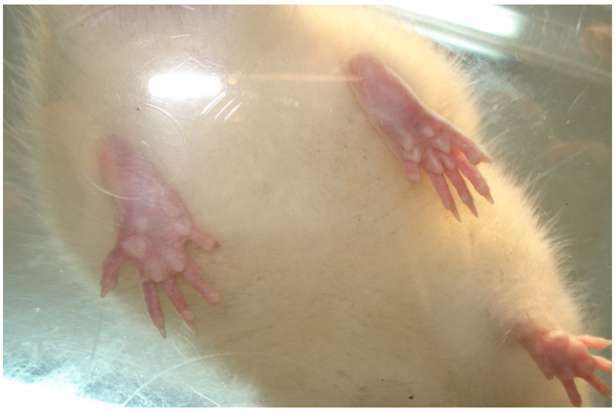


Fig. 2 – Position of the animal at rest, 4 months postoperatively, after end-to-side neurorrhaphy of the sciatic nerve (operated right hind limb)

During this period, potential complications, secondary to autotomy, were also recorded.

At 16 weeks postoperatively, the animals were re-anesthetized, and the sciatic nerve and gastrocnemius muscle were exposed both in the operated and the healthy hind limb, for the electromyographic study. The latency time and the amplitude of the compound muscle potential were determined both in the experimentally operated hind limb and the normal hind limb. The electrode used for stimulation was placed on the sciatic nerve, at its emergence (for the operated nerve this corresponds to the nerve segment above the suture), and the active electrode (used for recording) was placed on the gastrocnemius muscle; the distance between the stimulation electrode and the recording electrode was 25 mm. The nerve was stimulated with a 5 mV current at a frequency of 1 stimulus/s, so as to obtain maximum motor reaction (Fig. 3).

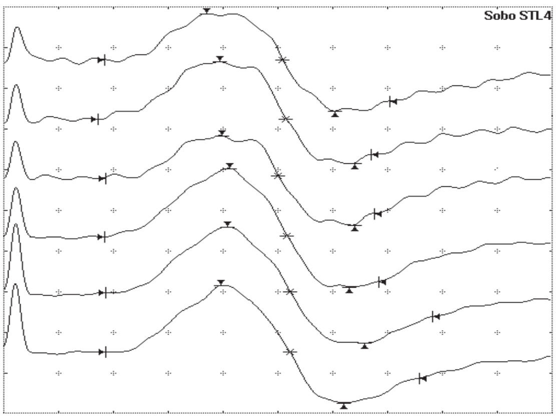


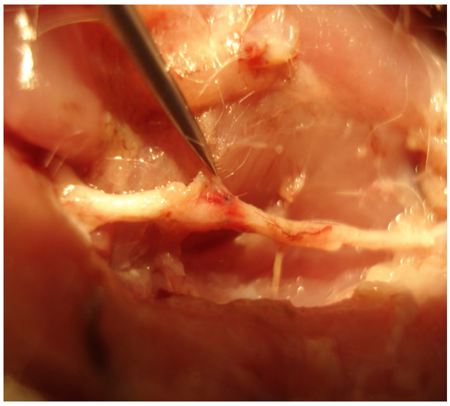
Fig. 3 – Electromyographic evaluation.

According to the protocol, the animals were sacrificed, by increasing the anesthetic dose, for the histomorphometric study, which assessed the number of axons, axon diameter, the thickness of the myelin sheath and macroscopic nerve changes in the suture.

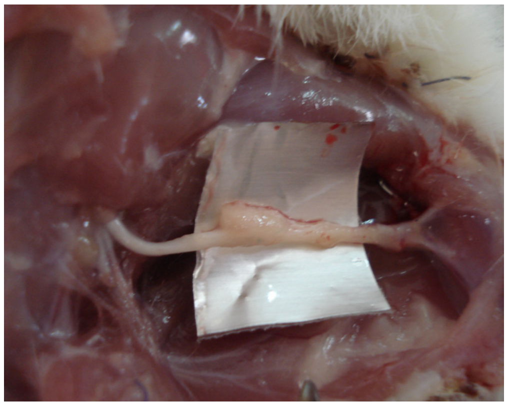
The sections were analyzed using a video camera and a semi-automated image analysis system Quantimed A. Seven locations were examined for each specimen, 4 at the nerve periphery and 3 in the nerve center. The total area examined in each nerve segment was 17.213 µm². The mean number of fibers analyzed in each segment was 232, varying from 121 to 389. Computerized histological measurements included the number of fibers/nerve area and their mean diameter, the thickness of the myelin sheath and the macroscopically visible fibrotic reaction both in the healthy nerve and in the operated nerve distal to the suture.

Minimal inflammatory or fibrotic reaction was marked with +, moderate reaction with ++, severe reaction with +++ and neuroma with ++++.

After the electromyographic study, the rats were re-anesthetized with a lethal dose of anesthetic. After the sciatic nerve was exposed, this was photographed in situ, and macroscopically visible abnormalities were recorded. Minimal inflammatory or fibrotic reaction was marked with +, moderate reaction with ++ (Fig. 4a), severe reaction with +++ and neuroma with ++++ (Fig. 4b).



a



b

Fig. 4 a – Moderate inflammatory reaction at the suture level
Fig. 4 b – Neuroma

d) Statistical processing

For statistical description and analysis of data, Microsoft Excel and SPSS 13.0 programs were used. Student’s t-test was employed to compare quantitative data between the studied paired samples. In order to investigate possible correlations between different quantitative variables, Pearson’s *correlation coefficient r* was used. In all situations mentioned above, the statistical significance threshold was considered α=0.05.

Results

Descriptive statistics of functional, electromyographic and histomorphometric parameters in group B are presented in Table I.

Table I

Correlations for the sciatic nerve: mean values, standard deviation

Parameters	Mean	Standard deviation
SFI - at 16 weeks	-62.304	39.2281
CMAP - operated limb	19.100	2.1048
NL - operated limb	1.5100	.08920
No. of distal fibers	12218.87	1354.552
Distal diameter	2.7187	.15734
Distal myelin	.6640	.08441
Fibrosis	3.53	.640

Table II shows a strong correlation, evidenced by morphometric determinations in the distal segment, between the number of fibers, axon diameter and the thickness of the myelin sheath. The more numerous the fibers, the thinner and less myelinized they were. The degree of fibrosis assessed macroscopically was correlated with no other determination. No correlation was found between the sciatic functional index (SFI), electromyographic determinations (CMAP, NL) and histomorphometric determinations (number of fibers, axon diameter, thickness of the myelin sheath and fibrosis), which were performed at the end of the follow-up period.

Discussions

Recovery after end-to-side neurorrhaphy is possible and can be explained by fiber regeneration from the lateral side of the donor nerve to the sectioned recipient nerve stump (Viterbo et al., 1994; Johnson & Zoubos, 2005).

Our study showed a highly significant correlation, evidenced by morphometric evaluation, between the number of fibers, fiber diameter and the thickness of the myelin sheath. The lower the number of fibers, the greater their diameter and the greater the thickness of the myelin sheath were (Gligor et al., 2008).

Our research demonstrated that end-to-side neurorrhaphy of the sciatic nerve is viable, able to generate a clinical response, to conduct electrical stimuli and allow axons to pass from the lateral side of the donor nerve to the distal end of the recipient nerve (Constantin et al., 2012; Urso-Baiarda & Grobelaar, 2006). It can be an alternative to direct end-to-end neurorrhaphy when this is not viable. The results obtained are in accordance with the studies of other authors in this area (Wang & Lineaweaver, 1999; Zhang et al., 1999; Rupp et al., 2007; Kanaya, 2002; Da Silva et al., 2007; Kovacic, 2007), as well as with our data demonstrated electromyographically and functionally (Gligor, 2009). The absence of correlation between functional, electrophysiological and histomorphometric determinations can be explained by the global reorganization of the nerve after transection and neurorrhaphy. The cross regeneration of axonal sprouts that can follow an erroneous pathway in the recipient nerve and their orientation errors can lead to a negative correlation between these methods. Only in the case of individual axons or nerves with very few fascicles is functional recovery similar to the histological and electromyographic result. Our study showed a highly significant correlation, evidenced by morphometric evaluation, between the number of fibers, axon diameter and the thickness of the myelin sheath. The lower the number of fibers, the greater their diameter and the greater the thickness of the myelin sheath were. The absence of correlation between the three evaluation methods (functional, electrophysiological and histomorphometric) supports that these should be considered separately by therapeutic studies. Our data are in agreement with literature data (Pessina Gasparini et al., 2007; De Medinaceli, 1995; Martins et al., 2006).

Conclusions

1. When the proximal nerve stump is absent, end-to-side neurorrhaphy is a real option, only when it is supported by an active nerve segment.
2. No significant correlation is evidenced between clinical, electrophysiological and histomorphometric assessment methods, which show different aspects of regeneration and should be considered separately by therapeutic studies.

Table II

Correlations in group B; Pearson correlation coefficients

Parameters	Statistical index	SFI 16 weeks	CMAP operated limb	NL operated limb	No. of distal fibers	Distal diameter	Distal myelin	Fibrosis
SFI16	Pearson correlation	1	-.307	-.300	.314	-.205	.085	.172
	Significance	–	.266	.277	.254	.463	.763	.539
CMAP in the operated limb	Pearson correlation	-.307	1	.402	.235	.041	-.272	-.090
	Significance	.266	–	.138	.399	.886	.327	.749
NL in the operated limb	Pearson correlation	-.300	.402	1	.251	-.211	.055	.250
	Significance	.277	.138	–	.367	.451	.846	.368
Number of fibers/ mm^2 in the operated limb	Pearson correlation	.314	.235	.251	1	-.842(**)	.839(**)	.195
	Significance	.254	.399	.367	–	.000	.000	.487
Mean distal axon diameter	Pearson correlation	-.205	.041	-.211	-.842(**)	1	-.852(**)	.142
	Significance	.463	.886	.451	.000	–	.000	.613
Distal myelin	Pearson correlation	.085	-.272	.055	.839(**)	-.852(**)	1	-.280
	Significance	.763	.327	.846	.000	.000	–	.312
Fibrosis	Pearson correlation	.172	-.090	.250	.195	.142	-.280	1
	Significance	.539	.749	.368	.487	.613	.312	–

Conflicts of interests
Nothing to declare.

Acknowledgments
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