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ELECTROPHYSIOLOGIC ASSESSMENT OF REGENERATION IN RAT SCIATIC NERVE REPAIR USING SUTURE, FIBRIN GLUE OR A COMBINATION OF BOTH TECHNIQUES

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ABSTRACT - We evaluated the repair of seccioned rat sciatic nerve by the comparison of electrophysiologic parameters. The repair was effected with suture (group A), fibrin glue (group B) or a combination of both techniques (group C). The amplitude, latency and conduction velocity of the motor and nerve action potentials were assessed before the nerve section and at reoperation after 24 weeks. There was no difference between the groups when the nerve action potential was evaluated. Rats of group B presented better results than those of group A ($p<0.05$) when latency and the nerve conduction velocity assessed at the reoperation, and the ratio between the conduction velocity at the reoperation and before the nerve section in the motor action potential evaluation were measured. Animals of group C presented better results than those of group A when the ratio between the conduction velocity of motor action potential at the reoperation and before the nerve division was considered ($p<0.05$). No difference between groups B and C was found. We conclude that repair using fibrin glue presented better results than suture following transection of sciatic nerve when the motor action potential was evaluated in the rat experimental model.

KEY WORDS: fibrin glue, nerve regeneration, suture, sciatic rat nerve.

Avaliação eletrofisiológica da eficácia de três tipos de reparo após a secção do nervo ciático do rato

RESUMO - Foram comparados os parâmetros obtidos na avaliação eletrofisiológica do potencial de ação do nervo e do potencial de ação motor antes e após 24 semanas do reparo no nervo ciático do rato previamente seccionado no lado direito com a utilização de sutura (grupo A), adesivo de fibrina (grupo B) ou uma combinação das duas técnicas (grupo C). Não houve diferença entre os grupos na avaliação do potencial de ação do nervo. Quando consideradas a latência e a velocidade de condução mensurados na reoperação e a razão entre a velocidade de condução medida na reoperação e o mesmo parâmetro antes da secção do nervo, durante a mensuração do potencial de ação motor, os animais do grupo B apresentaram melhores resultados em relação aos do grupo A ($p<0,05$). Os animais do grupo C apresentaram melhores resultados em comparação com os do grupo A quando considerada a razão entre a velocidade de condução medida 24 semanas do reparo e antes da secção do nervo durante a avaliação do potencial de ação motor. Conclui-se que os animais em que o reparo dos nervos foi realizado com o adesivo de fibrina apresentaram melhores resultados em comparação com a sutura quando considerados os parâmetros obtidos na mensuração do potencial de ação motor.

PALAVRAS-CHAVE: adesivo de fibrina, regeneração nervosa, sutura, nervo ciático do rato.

When a nerve is transected the continuity between the two stumps may be reestablished by different techniques. Although direct suturing of the nerve is considered the standard procedure, it can be difficult due to reduced nerve caliber and sometimes can cause inflammatory reaction impairing the axonal regeneration[1]. The repair with fibrin glue is an alternative to the conventional suture technique, although there is no definitive experimental evaluation of the two techniques[1-13].

In this study we compared the parameters obtained in the electrophysiologic evaluation in three

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different repairs techniques after the section of the rat sciatic nerve, trying to define which one allows better nerve regeneration.

METHOD

This study was approved by the local Ethics Committee. Tirthy male Wistar rats, weighing between 260 to 355 g, were used. The whole surgical procedure was performed inside a Faraday's cage in order to reduce eventual electromagnetic interferences. Each rat was anesthetized intraperitoneally with diazepam and ketamine. The animals were placed in prone position and the right sciatic nerves were exposed through a dorsolateral incision. After the nerve exposure, we performed an electrophysiologic evaluation with nerve action potential (NAP) and motor action potential (MAP) measurements. This first evaluation was done to verify the nerve eletrophysiologic integrity. To reduce any possible interferences two grounded electrodes were installed. One electrode consisted of a DMF25 (25x0.30 mm - 30G - Medtronic) monopolar straight line needle which extremity was placed inside the muscle adjacent to the nerve. The other electrode was a stainless steel 316L wire with a helical extremity. This electrode involving the nerve and increases the contact area. The recording were performed with a two channels portable electromyograph (Medtronic, Keypoint® portable model) with the high frequency filter regulated for five KHz and the low frequency filter regulated for two Hz. An electric monofasic stimulus were applied to the nerve in a single square pulse of 0.04 millisecond (msec). For PAN evaluation the bipolar stimulating and recording electrodes were positioned under the sciatic nerve proximal and distal to the repair site. The distance between the two bipolar electrodes was 2 centimeters (cm). A 1-msec supramaximal stimulus was applied to generate an action potential. From the recording of this initial potential (NAP1), the latency (LATN1) and the amplitude (AMPN1) were measured and the conduction velocity (CVN1) was calculated.

After NAP1 evaluation, the MAP (MAP1) was then measured. The recording electrode was left, the grounded electrodes and stimulus electrode were maintained in the same position as described for NAP1 record. The recording electrode, a DCF25 (25 X 0.30 mm - 30G - Medtronic) coaxial needle, was positioned in the gastrocnemius muscle through a percutaneus puncture. The distance between the stimulus and recording electrodes was 3 cm. From the motor potential recording, the latency (LATM1) and the amplitude (AMPM1) of MAP1 were measuredand the conduction velocity (CVM1) was calculated. After this initial evaluation, with the aid of an operating microscope, the nerve was dissected from its surrounding tissue and transected with microscissor half way between its origin and its first division. The next step was the immediate nerve repair.

The animals were distributed into three equal groups according to the operative procedure. In group A, the neurotomy was repaired by a microsurgical four-stitch epineural technique using monofilament 10-0 nylon. In group B, fibrin glue (Beriplast®, Aventis) was applied over the anterior epineural surface of the stumps. The nerve was gently turned to expose its posterior surface where the fibrin glue was again applied. The fibrin glue used in our study is a fibrinogen-based mixture with a two-component sealant. The first component consist of fibrinogen, factor XIII and bovine aprotinin. The second portion contains a mixture of thrombin and calcium chloride. Before application, the components were mixed and the resulting compound was immediately instilled in the epineurium. The nerve ends were maintained close together for three minutes to allow the stabilization of the fibrin glue. In group C the combination of the two previous techniques was performed. A single 10-0 monofilament nylon suture was associated with the application of fibrin glue over the opposite side.

The repairs were always done by the same investigator. After the wound closuring, each animal was maintained in a separated cage, fed and watered *ad libitum.* Each animal was numbered to avoid that the examiner knew which type of repair was applied.

A second electrophysiologic evaluation was carried out 24 weeks after the initial surgical procedure. The animals were anesthetized and the sciatic nerves were again exposed. The final nerve action potential (NAP2) and final motor action potential (MAP2) were obtained. The latency (LATN2), amplitude (AMPN2) and conduction velocity (CVN2) of NAP2 and the latency (LATM2), amplitude (AMPM2) and conduction velocity (CVM2) of MAP2 were evaluated. The ratio between the initial and final amplitudes of every potential in percentage (%AMPN and %AMPM) and the ration between the initial and final conduction velocity of every potential in percentage (%CVN and %CVM) were calculated. After electrophysiologic evaluation the animals were killed by an overdose of sodium pentobarbitol administered intraperitoneally.

The results are presented as the means ± standard deviation. The parameters were compared by non-parametric analysis of variance (ANOVA). The use of ANOVA was supplemented by Tukey's or Duncan's statistical tests when necessary. Statistical results were discussed at a significance level of 5 %.

RESULTS

Nerve action potential – Results of NAP analysis are shown in Table 1. The LATN2 results in group B were better when compared to group A (Tukey method, $p<0.05$) (Fig 1). Analysis of the other electrophysiologic data did not show any statistical differences between the three methods.

Table 1. Results of nerve action potential evaluation, including the p value.

	A	B	C	*p*
LATN1 (msec)	0.26 ± 0.10	0.30 ± 0.16	0.24 ± 0.08	0.508
LATN2 (msec)	0.41 ± 0.20	0.34 ± 0.15	0.33 ± 0.10	< 0.05
CVN1 (m/s)	86.07 ± 27.02	82.64 ± 35.94	92.18 ± 28.63	0.783
CVN2 (m/s)	59.55 ± 26.19	69.09 ± 28.85	66.24 ± 19.21	0.687
AMPN1 (mV)	1.29 ± 0.66	1.15 ± 0.59	1.14 ± 0.56	0.833
AMPN2 (mV)	0.37 ± 0.26	0.70 ± 0.52	0.45 ± 0.33	0.167

AMPN1, initial nerve action potentials amplitudes; AMPN2, final nerve action potentials amplitudes; LATN1, initial nerve action potentials latencies; LATN2, final nerve action potentials latencies; msec, millisecond; m/s, meters per second; mV, millivolt; CVN1, initial nerve action potentials conduction velocities; CVN2, final nerve action potentials conduction velocities.

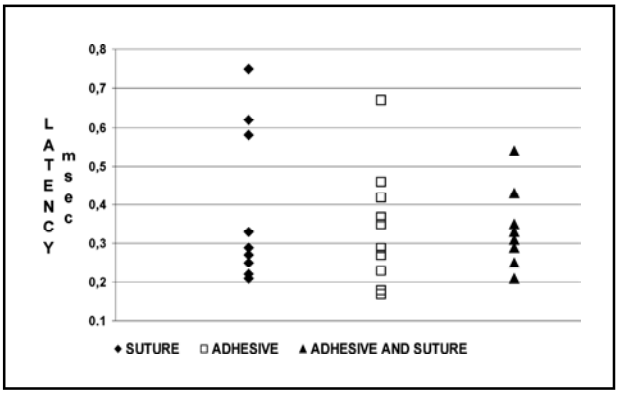

Fig 1. Graph showing latencies values of nerve action potentials in the three groups of nerve repair.

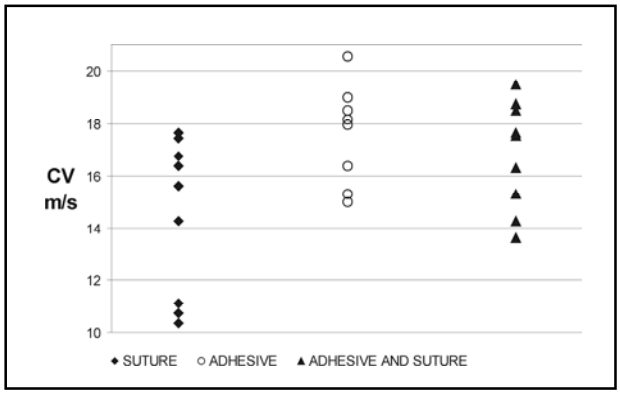

Fig 2. Graph showing conduction velocity values of the motor action potential in the three groups of nerve repair.

Table 2. Results of motor action potential measurement and the p value.

	A	B	C	p
LATM1 (ms)	1.48 ± 0.17	1.51 ± 0.16	1.67 ± 0.38	0.602
LATM2 (ms)	2.13 ± 0.48	1.74 ± 0.19	1.85 ± 0.25	< 0.05
CVM1 (m/s)	20.47 ± 2.34	20.04 ± 2.20	18.85 ± 4.27	0.602
CVM2 (m/s)	14.71 ± 2.91	17.44 ± 1.85	16.51 ± 2.19	< 0.05
AMPM1 (mV)	5.12 ± 3.23	5.15 ± 1.77	4.65 ± 1.33	0.719
AMPM2 (mV)	4.20 ± 2.48	4.95 ± 2.01	4.08 ± 1.69	0.603

AMPM1, initial motor action potentials amplitudes; AMPM2, final motor action potentials amplitudes; LATM1, initial motor action potentials latencies; LATM2, final motor action potentials latencies; msec, millisecond; m/s, meters per second; mV, millivolt; CVM1, initial motor action potentials conduction velocities; CVM2, final motor action potentials conduction velocities.

Motor action potential – Results of MAP analysis are shown in Table 2. The comparison of LATM2 and CVM2 values (Fig 2) between the groups showed a significant statistical difference between A group and the B group by Tukey method ($p < 0.05$). In the evaluation of %CVM, was identified a statistically significant difference between A group and the B group and between A group and the C group by Duncan method ($p < 0.05$). The comparison of the other parameters presented no statistical significant differences, included %CVM and %AMPM.

DISCUSSION

The analysis of the alterations in the electrophysiologic parameters before and after the repair showed modifications occurring in a similar way in the different groups, except for LATN2, VCM2 and %VCM. In the evaluation of these parameters a significant difference was identified between groups A and B for the three parameters and between groups A and C also for the %VCM.

These parameters were direct or indirectly related with the CV and the improvement probably was the consequence of a more effective myelination in the regenerated axons, mostly in the group B. In a serial electrophysiologic evaluation after section and repair of rabbit sciatic nerve using fibrin glue or suture, Moy et al.[4] found that CV recovery was superior to amplitude recovery at the same analysed period. In that study, the CV recovery in the animals whose sciatics nerves were submitted to repair with fibrin adhesive was 97 to 98 % of the initial CV while the amplitude recovery was 40 %.

Few studies in the literature performed an electrophysiologic evaluation protocol similar to the one used in this one. The results obtained in the amplitude and CV MAP before the nerve section were similar to the obtained by He et al.[14]. Only two studies, evaluating the effectiveness of the fibrin glue used to nerve repair, analysed MAP. The methodology and numerical results of the one study was not informed[5]. The results of MAP latency in our study were similar to that obtained by Inalöz et al.[10].

Smahel et al.[15] compared the electrophysiologic parameters after rat sciatic nerve repair using suture or fibrin glue. The NAP latency, show on a graph, was about three times smaller after six months in comparison to the value obtained after three weeks. In the same study, the value of the NAP amplitude was about twelve to sixteen times superior after six months in comparison to the value obtained after three weeks. Although the control group was constituted only by four animals, the results for %CVN and %AMPN is very similar to ours.

In only two articles the exact values of the NAP parameters were informed. In the study published by Ratto et al.[16] the latency in the sutured-nerve group - 0.69 msec - was larger than our results. This parameter was eight times larger than those obtained in our study in fibrin glue repair group. In the study of Maragh et al.[7], the results were three times larger than our results.

The difference observed between described PAN values in these studies and the results of our study could be justified by the used observation period. Two months seems to be enough time for the axons to cross the repair and reach end-organs. However, the fibers maturation, which includes myelination, may needs a lengthier period to be completed. Moreover, the presence of polyneuronally innervated muscular fibers, which occurrence reduces the final efficiency of the regeneration and consequently its pulse transmission, reduces in a progressive way after the lesion[17]. In this way, by the end of a prolonged observation period as we used, most remaining fibers would present a better quality in comparison to the available fibers in shorter observation periods.

In conclusion, in our study the experimental nerve repair with fibrin glue presented better results in comparison to the suture when motor action potential evaluation was considered. There was no difference between the repair methods when the nerve action potential evaluation was analyzed.

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