EXPERIMENTAL NEUROLOGY

The embracing end-to-side neurorrhaphy in rats

A neurorrafia término-lateral abraçante em ratos

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ABSTRACT

PURPOSE: Compare two new methods with the traditional end-to-side neurorrhaphy.

METHODS: Rats were divided into four groups. In A-L group the peroneal nerve was sectioned and the distal stump was connected to the lateral of the tibial nerve (donor) with two 10-0 nylon points. In A-R group two perineurium flaps embraced the donor nerve. In the B-R group a suture embraced the donor nerve. Group B-L was the control. After six months tibial cranial muscle mass and morphometry of the distal stump of the peroneal nerve were evaluated.

RESULTS: Muscle mass in groups A-R, A-L and B-R were lower than B-L group (p<0.0001) an equal between themselves (p>0.05). Groups A-R, B-R and A-L had a lower number of nerve fibers when compared with B-L (p=0.0155, p=0.016, p=0.0021).

CONCLUSION: The three types of neurorrhaphy showed no differences related to muscle mass and number of nerve fibers suggesting that the embracing with a single suture has great potential due its simplicity and usefulness in deep areas.

Key words: End-to-side neurorrhaphy. Embracing suture. Rats.

RESUMO

OBJETIVO: Comparar dois novos métodos com o método tradicional da neurorrafia término-lateral.

MÉTODOS: Os ratos foram separados em quatro grupos. No grupo A-E o nervo peroneal foi seccionado e o coto distal foi suturado à lateral do nervo tibial com dois pontos de nylon 10-0. No grupo A-D duas abas de epi-perineuro abraçaram o nervo doador. No grupo B-D foi realizada sutura com um único ponto abraçando o nervo doador. O grupo B-E foi o controle. Após seis meses foram observados massa do músculo tibial cranial e morfometria do coto distal do nervo peroneal.

RESULTADOS: Foi encontrada menor massa muscular nos grupos A-D, A-E e B-D quando comparados com o grupo B-E (p<0.0001) e mesma massa quando comparados entre si (p>0.05). Os grupos A-D, A-E e B-D apresentaram menor número de fibras nervosas quando comparados ao grupo B-E (p=0.0155; p=0.016; p=0.0021) e mesmo número quando comparados entre si.

CONCLUSÃO: Os três tipos de neurorrafia não apresentaram diferenças relacionadas à massa muscular e número de fibras nervosas sugerindo que a sutura abraçante com apenas um ponto apresente grande potencial em áreas cirúrgicas mais profundas.

Introduction

Since Viterbo et al. introduced the End-to-Side Neurorrhaphy (ETS) concept without lesion in the donor nerve, many studies were published.

When there is the proximal and distal stump after a nerve section it is possible to perform the surgical repair with the End-to-End Neurorrhaphy (ETE). In this process there is the union of the endoneurium conduits, making the nerve fibers regeneration easier.

In many clinical situations the proximal stump is not available, making the end-to-end neurorrhaphy impossible. In these cases, an option is the end-to-side neurorrhaphy, introduced by Viterbo et al., without lesion of the donor nerve. In experimental studies in rats, the peroneal nerve was sectioned and its distal stump was sutured to the lateral face of the intact tibial nerve, with and without the epi-perineurium removal. The proximal stump was put away and buried in the subjacent muscle. It was noticed growth of the axons from an intact nerve into the distal stump of a receptor nerve. For the first time they obtained muscle reinnervation without lesion of the donor nerve. The importance of this study was that any nerve can be a potential axonal donor. Later studies proved these initial findings.

Today we know that after some months of ETS, there is the absorption of the conjunctiva layers and lateral sprouting of axons coming from the intact nerve to inside of the receptor nerve, maybe due to the liberation of enzymes or nerve growth factors, still unknown. The direct contact between the donor and receptor nerve seems to be a prerequisite to the reinnervation, because it allows the interaction of the Schwann cells between the two nerves, maybe increasing the production of growth factors, inducing and leading to the axonial sprouting inside the sectioned nerve.

Currently the ETS is performed with two to four suture stitches in the lateral surface of the donor nerve. This is time consuming and a more delicate procedure. In an attempt of simplify this technique, two simple methods were tested, the “embracing” with epineurium flaps or just one “embracing” stitche.

The aim of this study was to compare two new methods to perform the ETS with the traditional one.

Methods

Twenty Wistar rats were divided between two groups, A and B. Each side was considered a sub-group, right (R) and left (L), constituting the groups A-R, A-L, B-R and B-L.

The rats had their mass measured and anesthetized with ketamine and xylazine at a dose of 70 mg/kg and 30 mg/kg respectively, intramuscularly. The posterior members had a 2 to 3 centimeters longitudinal incision, compromising skin and subcutaneous with posterior section of the biceps femoris muscle. After that, the procedure was performed, according to the experimental group.

In the group A-L a traditional end-to-side neurorrhaphy (TETS) was performed. The peroneal nerve was sectioned and the distal stump was sutured to the lateral surface of the tibial nerve with two 10-0 nylon stitches (Figure 1).

In the group A-R an embracing end-to-side neurorrhaphy with epi-perineurium flaps (ETSF) was done. The extremity of the distal stump of the peroneal nerve was divided longitudinally in two parts and the axons were removed, resulting in two epiperineural flaps. These flaps surrounded the tibial nerve and were fixed with one 10-0 nylon stitche (Figure 2).

In the group B-R an embracing end-to-side neurorrhaphy was performed with one embracing 10-0 nylon stitche (ETSS). The procedure was almost the same than in the group A-R, although without the epineurium flaps. A single nylon 10-0 point...
went through the epineurum of the peroneal nerve, passed behind
the tibial nerve, came back and passed in the front of this nerve,
and then went through the epineurum of the peroneal nerve again
like a loop, in a way that when fixed, this point approximated the
extremity of the peroneal nerve distal stump to the tibial nerve
(Figure 3).

FIGURE 3 – End-to-side neurorrhaphy with one embracing nylon stitche.

The group B-L (Control) was the normal control and did
not received any procedure (Figure 4).

FIGURE 4 – Normal control group did not received any procedure.
Sciatic nerve and its three branches: peroneal, tibial and sural nerve.

The epineurum of the tibial nerve were intact in all
groups. The proximal stump of the peroneal nerve was inverted,
buried in the subjacent muscle and fixed with one 5-0 nylon stitche.
The incision was sutured by plans with nylon 5-0.
The animals were housed in appropriate cages of the
Animal Laboratory of Surgical Technique and Experimental
Surgery, Department of Surgery and Orthopedics, with temperature
of 24 degrees (+/- 0.5), with water and ration ad libitum, respecting
the 12 hours dark/clear cycle.

After six months the rats were sacrificed and the cranial
tibial muscle and the distal stump of the peroneal nerve were
harvested. The muscles had their mass measured and the nerves
were submitted to fibers counting.

For statistic analysis, since the variable answers did
not show adherence to the normal distribution of probabilities,
the Mann-Whitney non-parametric technique was used. All the
comparisons were performed with the significance level of 5%.

Results

Table 1 and Figure 5 refers to the average of the cranial
tibial muscle mass. All groups were different from the control but
not between themselves.

TABLE 1 - Mean and standard deviation of the cranial
tibial muscle mass.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETSF</td>
<td>0.253</td>
<td>0.134</td>
</tr>
<tr>
<td>TETS</td>
<td>0.353</td>
<td>0.174</td>
</tr>
<tr>
<td>ETSS</td>
<td>0.392</td>
<td>0.161</td>
</tr>
<tr>
<td>Control</td>
<td>0.811</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Table 2 and Figure 6 refers to the average number of
nerve fibers per microscopic field of $0.175 \text{ mm}^2$. The experimental
groups also showed lower results from the control group, but not
between themselves.

TABLE 2 - Mean and standard deviation of fibers
number per microscopic field.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETSF</td>
<td>81.00</td>
<td>40.91</td>
</tr>
<tr>
<td>TETS</td>
<td>81.83</td>
<td>33.33</td>
</tr>
<tr>
<td>ETSS</td>
<td>82.90</td>
<td>20.06</td>
</tr>
<tr>
<td>Control</td>
<td>124.30</td>
<td>28.08</td>
</tr>
</tbody>
</table>
In 1895 Ballance (apud Ballance et al.\textsuperscript{10}) performed for the first time the suture of sectioned nerve to the lateral of a donor nerve, after cutting partially the donor. These lesions, determined by incision\textsuperscript{10} or by scarification\textsuperscript{11} in the lateral aspect of the donor nerve, allow the sectioned axons to grow inside the receptor nerves, providing, in fact, an end-to-end union between the endoneural tubes from both nerves.

These methods invariably showed functional disadvantage to the muscles innervated by the donor nerves. This observation leads Babcock\textsuperscript{12} to suggest the definitive abandon of the neurorrhaphy. After that, except for the Gatta\textsuperscript{13} and Pozzan\textsuperscript{14} publications, there was a gap in the literature, without any report about this subject. For these reasons plus the operative microscope and microsurgical techniques advent, the end-to-end neurorrhaphy (ETE) became the standard treatment for peripheral nerve lesion, and the end-to-side neurorrhaphy (ETS) was forgotten\textsuperscript{6}.

In 1992\textsuperscript{1-3}, with the introduction of the new concept of the end-to-side neurorrhaphy (ETS) without lesion on the donor nerve, new surgical possibilities appeared. Since the donor nerve doesn’t suffer any lesion, any nerve can be used as donor nerve.

During the end-to-end neurorrhaphy usually the suture is made with 8-0, 9-0, 10-0 monofilament nylon, or even thinner, like 11-0\textsuperscript{15}. It’s performed the epineural suture\textsuperscript{6-10}, the perineural suture, also called interfascicular or funicular\textsuperscript{15-18}, and even both associated in the same procedure, the epi-perineural suture. In this way, first the fascicles are sutured and then the epineurium\textsuperscript{6}.

The fibrin glue, already used in the ETE\textsuperscript{10-23}, can also be used in the ETS\textsuperscript{4}. An important disadvantage of this method is it high cost\textsuperscript{28}. However it’s use became very interesting, especially in some situations where the nerve to be repaired are deep, making it more difficult to do the sutures.

In this study it was tried a most simple alternative, that could also be applied in the depth. The result of this study showed that both proposed new techniques, epineurium flap and embracing suture were similar to the traditional ETS.

In the embracing suture it is obvious that the suture can’t be neither very tight, because it would cause stenosis in the donor nerve, nor very floppy, because it wouldn’t provide appropriate contact between both nerves. However the ideal tension still was not determined and it’s a subjective factor.

An interesting and contradictory aspect is the opinion of some authors who advocates lesion on the donor nerve, even minimum, like the ones caused by the needle during the suture in the end-to-side neurorrhaphy\textsuperscript{5,25-27}. These small lesions would be important to provide bigger contact between the nerves and also for the liberation of growth and neurotrophic factors, related to the development, maintenance and regeneration of the axonal sprouts\textsuperscript{28}. For this reason and also to reduce the dehiscence, some authors advocate the association of some anchorage microsurgical points in the ETS with fibrine glue\textsuperscript{29}.

In the present study no donor nerve lesion was done in both embracing ETS groups, and the axonal sprouting occurred similarly to the traditional ETS, where needle was used. Other authors, like Akeda et al.\textsuperscript{10} an Matsumoto et al.\textsuperscript{31} also noticed axonal regeneration with no donor nerve lesion, using a silicone chamber in “T” and without suture. These authors understand that the minimum lesion caused in the donor nerve can harm it, so it must be avoided\textsuperscript{31}.

The results obtained in the ETS with epi-perineural embracing flap and ETS with embracing suture support the theory that the two proposed techniques can be used in the ETS, even without harming the donor nerve.

The axonal sprouting exact mechanism through ETS still was not established\textsuperscript{4,30-32}. The macrophages, related to the Walderian regeneration, seem to be the responsible for the perineurium absorption, allowing the neurotrophins from the receptor nerve to initiate the axonal sprouting\textsuperscript{5,33}. This would explain the axonal sprouting after the embracing ETS, even without harming the donor nerve.

Liu et al.\textsuperscript{3} questioned the reproducibility of experimental studies in human begins. The authors affirm that the bigger perineurium, epineurium e endoneurium thickness from the human begins could prevent the axonal sprouting with techniques that doesn’t cut the donor nerve. However, Samii et al.\textsuperscript{32} reported success in a clinical case using ETS with fibrin glue, that, in the same way than the embracing from this study, didn’t have any...
lesion on the donor nerve.

Many experimental and clinical studies met good results with the ETS. Some, however, were not successful or the results were limited. Several technical aspects in the ETS performance are being studied and maybe can explain the bad results found by some authors.

In the present study was proved that the similar results between the three types of ETS, traditional, embracing with a single suture or two epineurum flaps. This leads us to believe in the big potential of the embracing ETS with a single suture because it’s simpler to be done, especially in deeper places.

**Conclusion**

The three types of neurorrhaphy showed no differences related to muscle mass and number of nerve fibers suggesting that the embracing with a single suture has great potential due to its simplicity and usefulness in deep areas.

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