INFLUENCE OF LASER RADIATION IN NERVE REGENERATION IN DIFFERENT TREATMENT SITES

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ABSTRACT

Objective: This study seeks to determine, through functional gait assessment in different irradiation sites, the influence of a low-intensity GaAsAl laser beam on an injury caused by crushing the peroneal nerve in rats. Methods: 53 rats were used, which were divided into six groups: normal, injured and untreated, injured and treated using placebo, injured and treated in the bone marrow, injured and treated in the nerve, and injured and treated in both (nerve and bone marrow). The peroneal nerve was crushed using a pair of tweezers, and subsequently treated with laser for 28 consecutive days. The functional gait evaluation analyzed the footprints, which were recorded with a video camera on an acrylic bridge in the preoperative period, and on postoperative days 14, 21 and 28, and assessed using PFI formula software. Results: In the functional gait evaluation, significant differences were found only on postoperative day 14. Conclusion: Based on the functional gait evaluation, low-intensity GaAsAl irradiation was able to accelerate and reinforce the process of peripheral nerve regeneration in rats on postoperative day 14, both in the bone marrow- and in the nerve-treated groups.

Keywords: Nerve regeneration. Crush syndrome. Laser therapy.

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INTRODUCTION

Several authors investigate the influence of the various therapeutic treatments, in the regeneration of rat sciatic nerve, such as electrical stimulation,[1] therapeutic ultrasound[2] and low-intensity laser that has received more attention in the last decade.[3] Low-intensity laser mechanisms are not fully understood, but some studies have attempted to explain the effects of their irradiation on biological tissue,[4,5] obtaining controversial considerations provided by different methodologies, such as dosage, wavelength, continuous or pulsed current, [4-6] irradiation site and treatment duration.[3]

The recovery of peripheral nerve injuries is studied mainly through electrophysiological and hystomorphometric parameters, which, although useful, do not keep track of functional recovery during treatment, which is important to quantify in a non invasive manner, and to reproduce the methods for a functional evaluation of nerve regeneration.[7] Therefore it might be better to use functional gait assessment rather than simply the electrophysiological and morphometric bases for axon growth and muscular innervation, provided the main interest of the survey is functional outcome.[8] The gait analysis has proved to be a safe method of functional assessment, monitoring the process from nerve lesion through to repair,[7,9] having a strong correlation with morphological evaluation.[1,9]

Low-intensity laser is usually irradiated at the site of the crush injury. Rochkind et al.[4] used laser irradiation at the root of the spinal cord, in the branch that corresponds to the sciatic nerve of rats (L2), and were able to observe an increase in neuron metabolism and an improvement in myelin production that serves to accelerate injured nerve regeneration. Anders et al.[3] explained that the effectiveness of low-intensity laser irradiation in peripheral nerve recovery can be increased if the corresponding segment of the spinal cord is also irradiated.

The aim of this study is to verify the influence of the low-intensity GaAsAl (830nm) laser beam on a crush injury of the common peroneal (common fibular) nerve of rats, through functional gait assessment at the different irradiation sites.

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MATERIAL AND METHODS

The study subjects were 53 male rats of the Wistar lineage aged approximately three months with mean weight of 220±30 grams. The animals were kept in collective cages, with five animals each, receiving commercial feed and water ad libitum, thus fulfilling the request of the Committee of Animal Experimentation Ethics of Faculdade de Medicina de Ribeirão Preto/USP, which approved this study. The animals were divided into six experimental groups, depending on the procedure performed:

GROUP 1: normal, uninjured and untreated (n=5);

GROUP 2: injured and untreated (n=10);

GROUP 3: injured and treated using placebo (n=8);

GROUP 4: injured and treated in the spinal cord, in the region that corresponds to the root of the sciatic nerve, by 4 points between vertebrae T12-T13-L1 along the segments of the spinal cord from L3-L6 (n=10);

GROUP 5: injured and treated on the right lower limb surrounding the scar, by 4 points (n=10);

GROUP 6: injured and treated on both; two points were divided for each region, whereas the points corresponded to L2 in the spinal cord and on the lower limb at the scar extremities (n=10).

The laser irradiation (Figure 1) was performed by commercial equipment of low-intensity Gallium Arsenate and Aluminum (GaAsAl) diode laser, known as Physiolux Dual, of the BIOSET brand, by the punctual transcutaneous method with contact for 28 days running and prepared with the following features: wavelength of 830nm, emitter power of 40mW, continuous mode, dose of 20 J/cm^2 and beam area of 3,464mm^2.

Surgery

After weighing, the animals underwent general anesthesia, administered intraperitoneally, of 4% Chloral Hydrate 10ml/kg of body weight, followed by care procedures such as trichotomy and antisepsis at the surgical site of the right lower limb to allow us to approach the common fibular nerve and to perform the crushing technique. The animals were positioned in ventral decubitus, with their rear and fore paws fixed in abduction, and the incision was made in the lateral region of the thigh, from the greater trochanter up to the lateral condyle of the femur. The musculature was separated by divulsion until it was possible to visualize the sciatic nerve and its three branches: common fibular, tibial and sural. Compression of the peroneal nerve covered a length of 5mm, with weight of 5kgf and crushing time standardized at 10 minutes. After crushing the nerve was placed in its bed, and the surgeon did not suture the muscles, but only the skin, using 3-0 nylon thread, and finalizing with hygiene and antisepsis care at the surgical site. The crushing of the peroneal nerve was performed using locking forceps specially designed for this purpose. These forceps produce a fixed static lesion of 5,000g, and were calibrated in advance in the Universal Testing Machine (EMIC®, model DL 10000).

Functional gait assessment

The footprints were analyzed for the functional assessment, recorded by the digital video camera, on an acrylic bridge with the following dimensions: corridor 43cm in length, 5.5cm in height and 8.7cm in width and a wooden hutch at the end of the course. The footprints were recorded using a Sony Handycam digital video camera recorder, and the captured images were analyzed by means of the AFNP – Functional Analysis of Peripheral Nerves software (Figure 2), which calculated the predetermined parameters for the functional gait assessment. Before the experiment the animals were made to walk on the acrylic bridge up to the wooden hutch in order to adapt. The footprints were obtained in the preoperative period, on the 14th, 21st and 28th days after the injury. The following parameters were measured in the footprints: E: experimental; N: normal; PL: print length; TS: total toe spread; IT: intermediate toe spread. The same data were entered in the formula of Bain et al.[10,] and provided the peroneal functional index (PFI):

$$PFI = 174.9 \times \left(\frac{EPL - NPL}{NPL} \right) + 80.3 \times \left(\frac{ETS - NTS}{NTS} \right) - 13.4$$

Statistical analysis

To attain the comparative goals they used the fixed effects linear model (random and fixed effects) that is employed in the data analysis where the answers of the same individual are grouped and the assumption of independence among observations in the same group is not adequate. For the use and adaptation of this model, it is necessary for the residue to have normal distribution with mean value zero and constant variance. When the assumption was not fulfilled, a transformation was used in the

Figure 1: *Schematic of the treated groups, showing the location of GaAlAs laser irradiation*

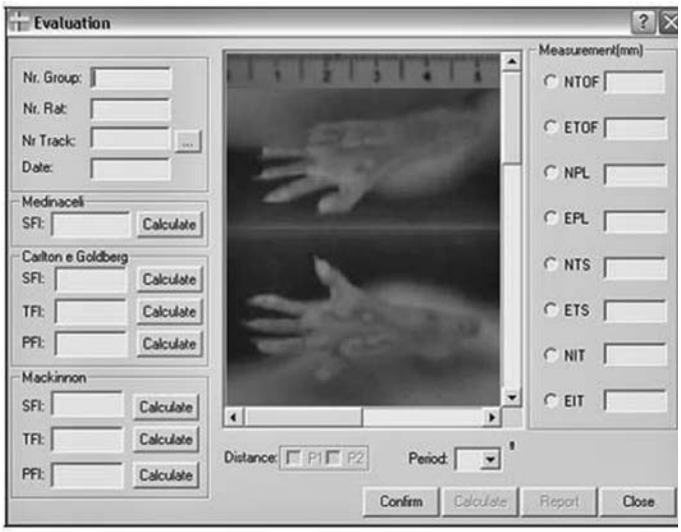

Figure 2: *Evaluation screen of AFNP (Functional Assessment of Peripheral Nerves) software.*

answer variable. The model was adjusted through the PROC MIXED procedure of the SAS® 9.0 software, considering the significance level of 5%.

RESULTS

The study was conducted with a total 200 footprint images in different periods. No statistically significant values were observed on postoperative days 21 and 28 in the functional gait assessment, when the 6 groups were compared and intercalated. Statistically significant differences were only found on the 14th postoperative day. (Table 1)

Group 2, injured and untreated (-44.29 ± 14.8), when compared with Group 4, treated in the spinal cord (-35.31 ± 10.31), and with Group 5, treated in the nerve (-32.77 ± 14.96), presented statistically significant differences with $p=0.04$ and $p=0.01$, respectively; Group 3, placebo (-44.31 ± 15.45), when compared with Group 5, treated in the nerve (-32.77 ± 14.96), presented statistically significant difference with $p=0.01$. (Figure 3)

Group 2, injured and untreated, and Group 3, placebo, presented similar mean values of -44.29 ± 14.8 and -44.31 ± 15.45, respectively. Yet when compared with Group 4, treated in the spinal cord (-35.31 ± 10.31), the first presented statistically significant difference ($p=0.04$) while the second did not present any difference ($p=0.06$) in the functional assessment of the 14th postoperative day.

The PFI in the preoperative period in all the groups ranged between the mean values; minimum of -8.68 ± 7.43 and maximum of -14.24 ± 6.32.

In the PFI formula the mean value in the functional gait assessment of the 14th postoperative day demonstrated a better recovery of the group treated in the nerve (-32.77 ± 14.96) than in the other groups treated, whereas the recovery of the group treated in the spinal cord (-35.31 ± 10.31) was better than the group treated in both (-37.62 ± 12.94). The same standard of difference among mean values in the functional gait assessment was maintained on the 21st postoperative day.

Table 1: *Comparisons for the variable PFI designating the 14th day post-operative*

Time	Group	Mean	Standard Deviation	Group	Mean	Standard Deviation	P Value
	1	–7.77	9.85	2	–44.29	14.80	<0.01
	1	–7.77	9.85	3	–44.31	15.45	<0.01
	1	–7.77	9.85	4	–35.31	10.31	<0.01
	1	–7.77	9.85	5	–32.77	14.96	<0.01
	1	–7.77	9.85	6	–37.62	12.94	<0.01
	2	–44.29	14.80	3	–44.31	15.45	0.97
	2	–44.29	14.80	4	–35.31	10.31	0.04
14th day	2	–44.29	14.80	5	–32.77	14.96	0.01
	2	–44.29	14.80	6	–37.62	12.94	0.14
	3	–44.31	15.45	4	–35.31	10.31	0.06
	3	–44.31	15.45	5	–32.77	14.96	0.01
	3	–44.31	15.45	6	–37.62	12.94	0.15
	4	–35.31	10.31	5	–32.77	14.96	0.56
	4	–35.31	10.31	6	–37.62	12.94	0.66
	5	–32.77	14.96	6	–37.62	12.94	0.31

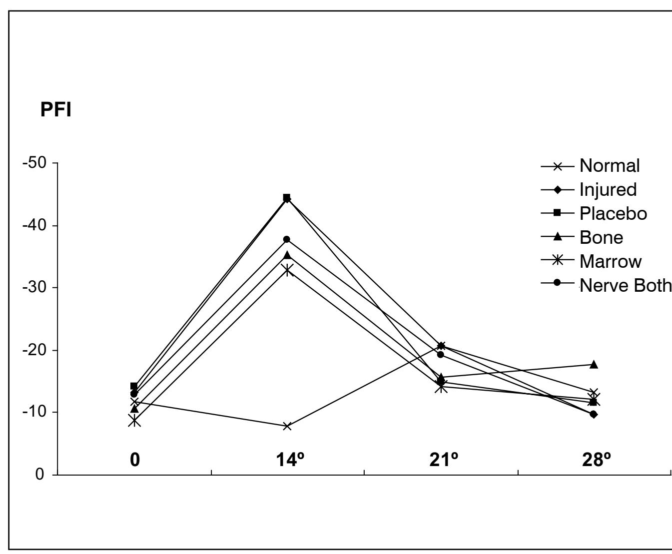

Figure 3: *Peroneal functional index (PFI) in accordance with the behavior of groups in relation to time.*

DISCUSSION

The rat was the animal chosen for the performance of the experiments due to the fact that they entail a low cost and are easy to handle, with peripheral nerves that resemble those of humans, as well as the physiology and the biological processes involved in regeneration. For these reasons the rat is the animal most frequently used in peripheral nerve regeneration studies.[8,11]

The surgical procedure, for the lesion of the common peroneal nerve using the forceps, is easy to learn without the need for microsurgical equipment, and lasted 20 minutes on average for each animal. The crushing injury was produced in all the animals by fixed load crush forceps (5kgf), the same used by Monte et al.[12] to make the lesions homogenous. This method does not provoke nerve stretching, as the nerve does not need to be exposed, but is crushed in its own bed, thus ruling out another variable, that of nerve stretching.

After the peripheral nerve injury, several authors reported some complications such as articular contractures in paw flexion,[8] death due to anesthetic accidents, necrosis of the toes of the paw, death for no defined cause during treatment,[9] self-mutilation of the operated paws,[7] infections and suture dehiscence during treatment. These complications lead to the exclusion of the animal. In this study, five animals died in the postoperative period, probably due to an overdose of anesthetic, with no more cases of exclusion of animals on account of any other complication. We did not find any problems in the laser application as the animals behaved well during the treatment that lasted 40 seconds for each point, with a total 4 points per animal.

Treatment with low-intensity laser beams has been investigated by several authors that reported its benefits in nerve tissue regeneration. Laser irradiation promotes significant functional recovery,[13-15] increases the quantity of connective tissue (collagen), increases the number of fibroblasts at the site of the injury and that of myelinized axons, accelerating nerve conduction,[15] accelerates healing,[13,16] induces faster recovery from muscular trophism[13] and acts both in regeneration and in the prevention of nerve fiber degeneration.[17]

Rochkind et al.[17], Khullar et al.[6] and Gigo-Benato et al.[13] studied the influence of low-intensity laser, irradiated along the nerve path, on peripheral nerve injuries and found positive effects for nerve regeneration. In studying low-intensity laser irradiation in the spinal cord, in the region corresponding to the injured nerve, Rochkind et al.[4] reported an improvement in the recovery of the corresponding injured peripheral nerve, while Bagis et al.,[5] in conducting a study with the crushing of rat sciatic nerves, used the same irradiation site with the GaAs laser and declared that it is inefficient in the repair of nerve lesions.

In the study by Bagis et al.[5] two factors might have influenced the fact that no improvement was found in nerve regeneration, the probably short application time of the laser in seven consecutive days, and the pulsed emission of the low-intensity laser chosen for the study.

Anders et al.[3] proposed that the effect of low-intensity laser beams on peripheral nerve recovery can be increased if both the nerve path and the corresponding segment of the spinal cord are irradiated. Future studies investigated the influence of laser beams on nerve regeneration when irradiated at both sites, in the spinal cord and in the nerve, as well as the studies by Rochkind et al.[18] on rats and Rochkind et al.[15] on humans, which showed an improvement in nerve regeneration when compared with their respective placebos.

In our study we did not find any improvement in peripheral nerve regeneration through functional gait assessment in the group treated with GaAsAl's laser (830nm) in both sites, in the follow-up of the spinal cord and on the nerve path. This group did not obtain a statistically significant difference when compared with the other groups.

Functional gait assessment is the method most frequently used in our laboratory, in rat sciatic nerve investigations, showing a clear correlation between this and the assessment by nerve morphometry.[1,2,9]

The gait patterns developed in the injury of each nerve are foreseen by the basic grounds of anatomical principles. With the lesion of the common peroneal nerve, the extensors digitorum, the dorsiflexors and those which assist with foot eversion are denervated, causing the non-opposition of the toes and flexion of the paw that culminates in shortening of the print length. The distance between the intermediate toes is relatively unaltered due to the normal function of the intrinsic paw parts. Only a slight decrease of toe spread is noticed.[10]

The functional gait assessment in the preoperative period did not reach the value zero, as was expected, but rather oscillated around -10. Medonça et al.[1] also discovered the same oscillation.

On the 14th postoperative day the groups, treated in the spinal cord and treated in the nerve, exhibited an improvement in functional gait assessment when compared with the untreated injured group. And the group treated in the nerve presented an improvement in relation to the placebo group, which did not occur with the group treated in the spinal cord.

When compared, the group treated in the nerve presented a better mean value than the group treated in the spinal cord, which explains the fact that the group treated in the nerve presented statistically significant difference when compared with the placebo group and with the untreated injured group, while the group treated in the spinal cord presented statistically significant difference only when compared with the untreated injured group, although the placebo and untreated injured groups presented similar mean values.

The mean values of functional gait assessment also show the best result obtained with low-intensity laser treatment on the nerve path when compared with the results of the other treatments; furthermore, the outcome of treatment in the medullar segment corresponding to the nerve is better than the outcome of treatment in both places.

CONCLUSION

We concluded that low-intensity GaAsAl (830nm) laser irradiation was able to accelerate and potentialize the peripheral nerve regeneration process of rats on the 14th postoperative day, according to the functional gait assessment, both for the group treated in the spinal cord and for the group treated in the nerve.

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