ACTION OF THERAPEUTIC LASER AND ULTRASOUND IN PERIPHERAL NERVE REGENERATION

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ABSTRACT

Objective: To assess the efficacy of early therapeutic laser and ultrasound in the regeneration process of an injury in rats. Methods: We used 24 rats. Eighteen underwent surgery for sciatic nerve compression by a hemostat above the popliteal fossa. The animals were divided into three groups of six animals each. Normal control group. GI: Injured control without therapeutic intervention. GII: laser ArGaAl therapeutic intervention. GIII: therapeutic intervention of Pulsed Ultrasound. We begin therapeutic interventions 24 hours after injury, with daily applications for a period of fourteen consecutive days. Results: In assessing the girth of the muscles of the right they, the following average decrease (in mm) for each GI: 0.45, GII: 0.42, GIII: 0.40 In relation to travel time, both GII and GIII presented significant difference when compared to GI. In the final evaluation of the IFC, GII excelled in the GIII. As for the healing observed, a major great improvement was observed in GII and GIII. Conclusion: The results showed that nerve recovery was higher with the laser application. ***Level of evidence II, Therapeutic Studies – Investigation of the results of treatment.***

Keywords: Laser therapy, low-level. Ultrasonic therapy. Sciatic nerve. Nerve crush. Nerve regeneration.

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INTRODUCTION

We often observe traumatic peripheral nerve lesions, especially in the nerves that pass through the limbs. Injuries are usually caused by avulsion, compression, crushing, partial and total sectioning or stretching, resulting in the interruption of nerve impulses. This process can bring about loss or reduction of sensitivity and motor function in the innervated area, leading to countless nerve and muscle abnormalities.[1] Besides structural alterations in the muscle after nerve lesion, there are also metabolic alterations and gene expression of the musculoskeletal system such as the increase of acetylcholine receptors in the sarcolemma. Denervation causes an increase and proliferation of the extrajunctional acetylcholine receptors. This fact is reported as inducing axonal sprouting from the nerves, and is also a form of preparation for the formation of a new neuromuscular junction.[2]

We can consider the nature and level of the lesion, the type and diameter of the injured nerve fibers, age, denervation time and other individual variables to be factors that influence the regeneration of the affected nerve fiber. According to Seddon[2] the classification of peripheral nerve lesions is based on the degree of rupture of the internal structures of the peripheral nerve, which is correlated with the recovery prognosis, being divided into neuropraxia, axonotmesis and neurotmesis. Although the peripheral nerves regenerate and consequently restore the lost nerve functions, it is known that morphologic and functional recovery after nerve lesion is rarely complete and perfect, even when modern and sophisticated reconstruction techniques are applied.[1] Due to these factors, functional recovery after nerve lesion occurs in a unique manner, whereas in some cases it may be unsatisfactory.[3] It is therefore understood that in addition to surgical techniques, it is also necessary to use physical means of assisting in the better prognosis of functional rehabilitation.[4] Among the resources commonly used in physiotherapy geared towards the regeneration of peripheral nerve lesions, we can cite electrical, ultrasound and low-level laser stimulation, seeking an early return of the patient's functionality. We must remember the extreme importance of post-injury rehabilitation, thus guaranteeing that patients will have sufficient recovery of neuromuscular function. Electrotherapy is important to speed up the promotion of the improvement of function and of peripheral nerve regeneration.[4,5] Thus the aim of this study was to verify the benefits of low-level laser therapy and therapeutic ultrasound in nerve regeneration as well as the best proposal of weekly intervention after sciatic nerve compression in rats.

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

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METHODOLOGY

The experiment was developed at the multidisciplinary research laboratory of Centro Universitário do Planalto de Araxá and was previously approved by the research ethics committee of this teaching institution (Protocol no. 18684/214). The study subjects were 24 adult female Wistar rats, with average weight of 182g, from the vivarium of Centro Universitário do Planalto de Araxá. The animals were kept grouped in restraint cages, with the maximum number of three animals per cage, with unrestricted access to water and feed, under controlled environmental conditions throughout the experiment.

The animals were divided into a group that did not undergo surgical intervention (called normal control) composed of six animals and another group (subdivided into GI, GII and GIII), composed of 18 animals that underwent surgery for sciatic nerve lesion, through crushing.

The animals that suffered injury were divided into 3 study subgroups.

Group I (Injured control): Six animals submitted to the surgical procedure for sciatic nerve injury, without therapeutic intervention.

Group II (Laser): Six animals submitted to the surgical procedure for sciatic nerve injury, which underwent therapeutic intervention with Aluminum Gallium Arsenide (AlGaAs) laser.

Group III (TUS): Six animals submitted to the surgical procedure for sciatic nerve injury, which underwent intervention with therapeutic ultrasound.

At first, the rats were weighed to calculate the dose of anesthetic, followed by the application of 0.2ml (fixed dose) of 2% aqueous solution of xylazine hydrochloride associated with 50 mg/ml ketamine hydrochloride, vol. = 0.1ml for every 100g. After checking the animal's state of consciousness (by pinching the interdigital folds), it was placed in the prone position, keeping the fore and hind paws in abduction, with the surgeon performing the trichotomy of the right hind paw. An incision was then made in the skin, starting below and medial to the greater trochanter and finishing close to the popliteal fossa, at the level of insertion of the ischiotibial muscles. The exposure of the sciatic nerve was accomplished after the separation of the semitendinosus muscle and of the rectus femoris muscle.

Using a pair of 540 mmHg hemostatic forceps, the above nerve was strangulated for 30 seconds. After this local asepsis was performed with iodized alcohol (70% alcohol + 10% iodine). The incision was closed with 4-0 mononylon thread accompanied by topic application of an antibiotic and anti-inflammatory (neotopic SM® spray).

All the animals underwent an analysis of the perimeter of the right and left hind thigh using a caliper (Starrett® series 721) before the surgical procedure. After the end of the experiment, all the animals once again underwent the same comparative analysis.

In the animals that did not undergo surgical intervention and for those that did, analog gait data collections were performed after the scheduled recovery period (24 hours) and on day 7 and 14. The data were collected on a walking track (walking in a single direction, towards the dark box at the end of the track and handling for the impregnation of the hind feet with ink). This track was made from MDF (medium density fiberboard, thickness of 1.5cm) and acrylic, measuring 52cm in length, 8.5cm in width and 14.5cm in height for the lateral protections, which was the only part made in acrylic. The dark box was also made of MDF with the following measurements: 30cm in length, 20cm in width and 14.5cm in height, with an opening of 8.5cm in the middle for the fitting of the track.[6]

For the gait analysis, the sciatic functional index (SFI) was calculated for the gait evaluation of all the animals. For the analogical analysis, the rats' hind paws were pressed lightly on a rubber stamp soaked in Indian ink. After this, the animal was positioned to walk along the walking track on which we deposited a strip of paper marked in millimeters. The paper strips were appropriately identified and left to dry for 12 hours. The footmarks printed on the paper strips were subsequently measured with a caliper (Starrett® series 721), and the respective values were introduced into a spreadsheet for obtainment of the SFI.

Summarily, the SFI values were obtained by the following formula:

$$SFI = -38.3 (EPL - NPL/ NPL) + 109.5 (ETS - NTS / NTS) + 13.3 (EIT - NIT / NIT) - 8.8$$

In this formula, EPL = experimental paw length (distance between the heel and 3rd toe); NPL = normal paw length (distance between the heel and 3rd toe); ETS = distance between the 1st and 5th toes of the experimental paw; NTS = distance between the 1st and 5th toes of the normal paw; EIT= distance between the 2nd and 4th toes of the experimental paw; NIT = distance between the 2nd and 4th toes of the normal paw. Positive values (above zero) characterize preserved/recovered motor function.[7] SFI values below zero show functional impairment, and close to -100 (minus one hundred) represent total functional loss.[8]

All the animals were submitted to the analysis of travel time, given in seconds, on the set course of the walking track before the injury, after the injury, one day after the injury and on the 7th and 14th days after treatment. The scanning of the animals' walking pattern was obtained using a Sony DSC-W70 video camera with 7.0 megapixels, positioned at a distance of 1 meter from the walking track. The data gathering contained lateral images of the rats covering the entire set course, from the start to the end of the walking track.

Therapeutic intervention was started with the animals from GII and GIII one day after the surgical procedure. These interventions occurred on 14 subsequent days and followed the pre-established parameters.

Group GII - Treatment: Laser therapy

The rats from group II were irradiated transcutaneously with AlGaAs laser (830nm, 40mW, Bioset), observing the specific technique for reducing reflection with density of 4 J/cm^2 starting 24 hours after the strangulation, for 14 days. The laser was applied to 2 parallel irradiation points on the surgical incision, always at the same times, with the assistance of two people keeping the animal immobile.

Group GIII: Treatment: Ultrasound irradiation.

The rats from group GIII were irradiated with the low-level pulsed ultrasound equipment Kroman KC 709M with the presence of ERA of 3.5cm^2 adapted to the coupler, adjusted to pulsed wave (1: 5, or 20%), in the frequency of 1 MHz and intensity of 0.4 W/cm^2, for 2 minutes with application of the head on the surgical incision and the use of ultrasound coupling gel, starting 24 hours after the strangulation, for 14 days, always at the same time and with the assistance of two people, keeping the animal immobile.

The data were tabulated and we applied the Student's t-test for comparison between control group and injured group (GI), with significance level of 5% ($p<0.05$). After this verification, the SFI, perimeter and travel time were submitted to statistical tests by comparative analysis (ANOVA) with Bonferroni posttest between the control group (group I), laser therapy group (group II - GII) and ultrasound group (group III - GIII) with significance level of 5% ($p<0.05$). The captured images were displayed through visual analysis.

RESULTS

In evaluating the transverse distance of the muscles of the right thigh of the animals that underwent surgery, before the surgical procedure and on the last day of evaluation, we obtained the mean subtracted values contained in Table 1.

In analyzing the travel time of the animals on the walking track, we obtained the values expressed in seconds. (Table 2 and Figure 1)

We initially noted an increase in the travel time of the injured control group (GI) when compared with the normal control group ($p<0.05$).

After the treatment, we noted reduced travel time in all the groups evaluated, although GII achieved a shorter travel time over the course of the evaluations ($p<0.05$).

The analog functional analysis was carried out with the uninjured animals and in all the groups studied on the 1st, 7th and 14th days after injury. The analysis was carried out numerous times with some animals, since in positioning the animal's paws on the pad to soak it in ink, this procedure also soaked the tail, leaving tracks on top of the footprints, rendering the recording process useless and entailing the necessity of a new data collection.

After the elimination of these artifacts, the SFI analysis was executed using the formula described by De Medinaceli et al.[8]

A statistically significant difference ($p<0.05$) was initially verified between the normal control group and the injured group (Table [3], Figure 2 and 3A and 3B).

The first SFI analysis, carried out 24 hours after the injury, showed 100% loss of motor function in all the animals from the groups after surgery, which meant that there was a considerable change in the rats' gait pattern. The footprints were long and narrow on the injured side in comparison to the normal side, because the animals used both heels and pulled in their toes for the intermediate weight-bearing phase. The rats were able to walk on their toes, but unable to transfer their weight to them. Moreover, the footprints also showed adduction of the toes, and the fall of the right paw could be seen during the swing phase of gait. In comparing the mean SFI values of groups GI, GII and GIII, we obtained the values represented in Table 4 and illustrated in Figure 4. It can be seen that both GII and GIII presented significant difference ($p<0.05$) when compared with GI, in the second and third evaluation, indicating the benefit of the two therapeutic procedures. In comparing groups GII and GIII, in the second evaluation we observed greater non-significant recovery of GIII ($p>0.05$). In the third evaluation, GII presented a better SFI than GIII ($p>0.05$). Figures 5 (A,B,C), 6 (A,B,C) and 7 (A,B,C) describe the results obtained in the 3 evaluations performed.

The recovery of the individual variables of SFI occurred simultaneously over the 14 days of experiment, showing an improvement in the gait pattern in each new analysis. The weight bearing of the heel gradually improved; the distance between 1st and 5th toes and between the 2nd and 4th toes gradually increased.(Table 5)

Table 1. *Representation of the analysis of the transverse size of the thigh muscles of the right hind leg.*

Groups	Decrease (in mm) of the right hind leg
GI	0.45
GII	0.42
GIII	0.40

Table 2. *Travel time values in seconds.*

	1st	2nd	3rd
GI (Control)	6.3	4.1	3.5
GII (Laser)	6.0	3.0	2.0
GIII (UST)	5.0	4.0	3.0

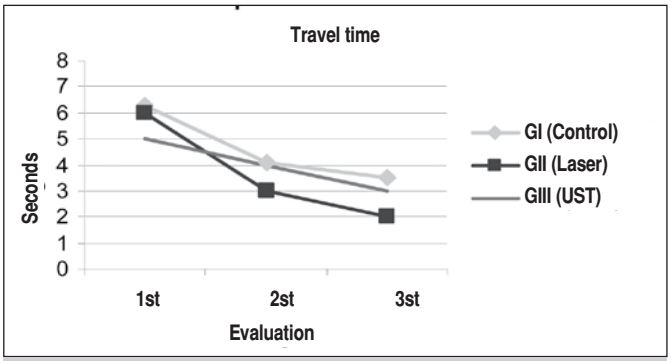

Figure 1. *Graphic representation of the travel time during the evaluations of the analyzed groups.*

Table 3. *SFI values of the normal and injured control groups (GI).*

Rat	Normal Control	Injured Control (GI)
Mean	11.21	-27.11
SD	10.24	3.34

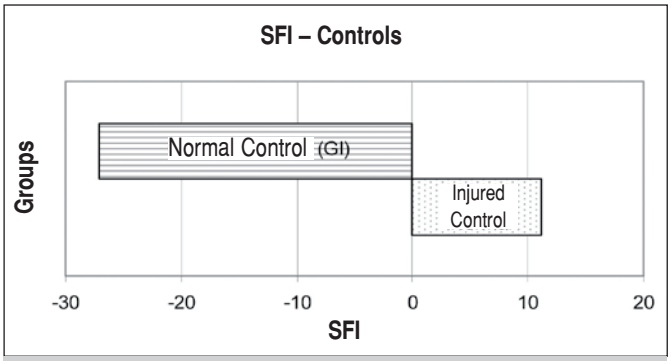

Figure 2. *Graphic representation of the SFI of the control groups.*
Source: Data from the Survey

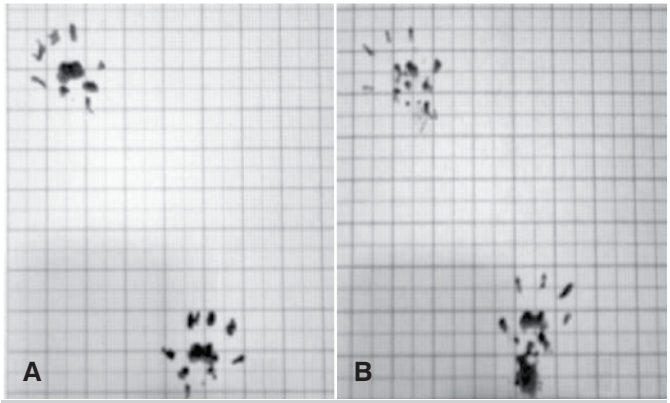

Figure 3A and 3B. *Representation of the footprints on the walking track of the normal control group A and B: injured control group.*
Source: Data from the survey

Table 4. *SFI of groups GI, GII and GIII during the evaluations (mean values).*

	Injured control (GI)	Laser (GII)	Ultrasound (GIII)
1st evaluation	-27.11	-26.46	-26.67
2nd evaluation	-25.9	-21.03	-19.43
3rd evaluation	-22.54	-5.37	-9.85

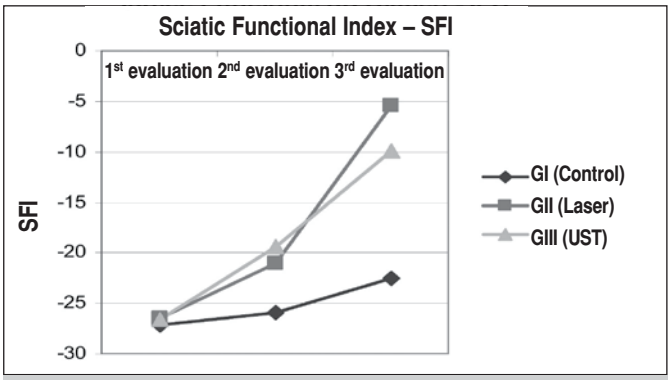

Figure 4. *Graphic representation of the mean values of SFI of the groups evaluated at 3 days of evaluation.*
Source: Data from the survey

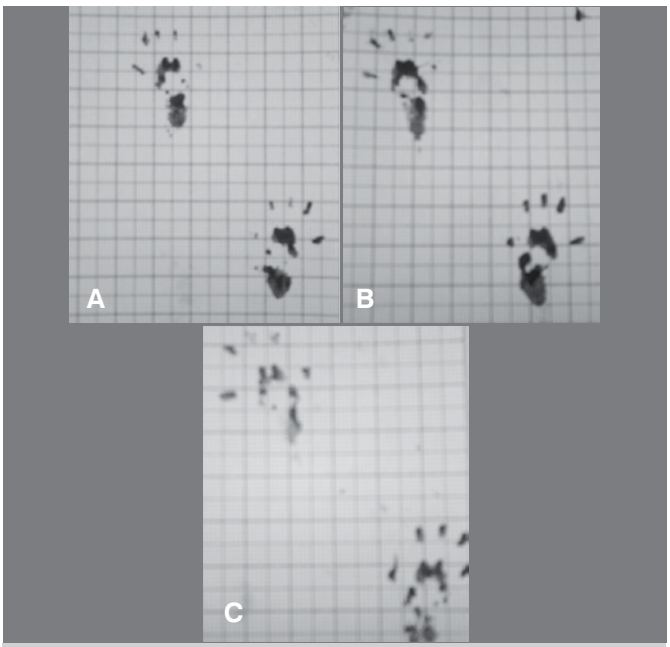

Figure 5 (A, B, C). *Representation of the footprints of groups GI, GII and GIII in the 1st evaluation.*
Source: Data from the survey

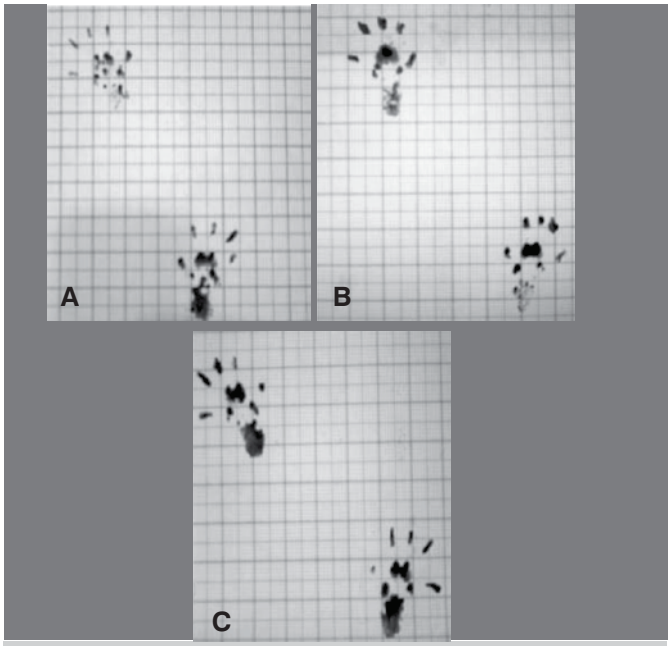

Figure 6. *(A, B, C).Representation of the footprints of groups GI, GII and GIII in the 2nd evaluation.*
Source: Data from the survey

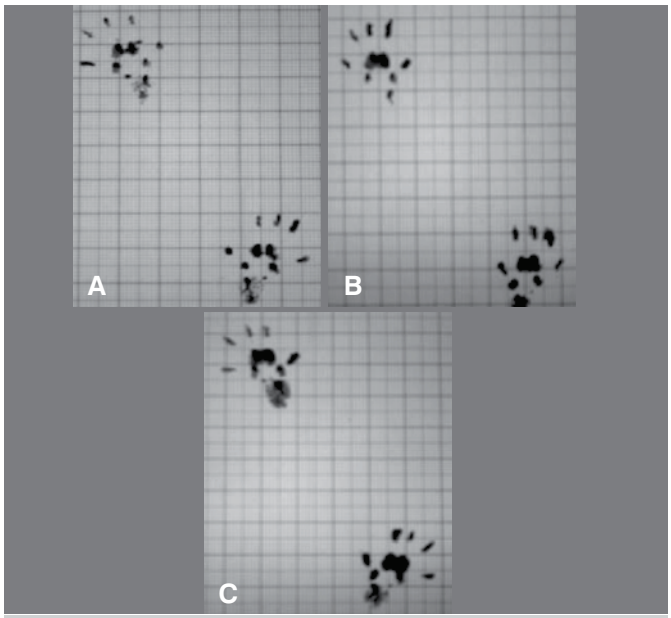

Figure 7 (A, B, C). *Representation of the footprints of groups GI, GII and GIII in the 3rd evaluation.*
Source: Data from the survey

DISCUSSION

In comparing the laser and ultrasound groups (GII and GIII) we evidenced a better response by GII in comparison to GIII in the travel time, SFI and healing at the end of the third evaluation. This fact seems to suggest that the laser generated more benefits in biological recovery when compared with the ultrasound. Among the effects of laser observed in clinical and experimental trials, we can cite the increase in nerve function, prevention of scab formation, boosting of neuronal metabolism and increase in myelin production capacity.[9] Since laser therapy is not an invasive procedure, its application is very beneficial to the ability to irradiate injured nerves.[10]

AlGaAs laser with 660Nm was adopted due to its low intensity and the wavelength that is widely used in clinical practice; moreover, this wavelength does not have a considerable number of previous studies on its effects in peripheral nerve regeneration.[11-13] In the clinic, low-level laser therapy employs doses from 1 to 4J/cm2, associated with output power between 10 to 90mW, and is widely used in various musculoskeletal lesions, as well as in painful and inflammatory processes.[10] Based on this fact the density of 4J/cm2 is justified in this survey. It is important to stress that this parameter is extremely variable in surveys on laser therapy in nerve regeneration.

Table 5. *Representation of the individual variables of SFI. EPL = experimental paw length (distance between the heel and 3rd toe); NPL = normal paw length (distance between the heel and 3rd toe); ETS = distance between the 1st and 5th toes of the experimental paw; NTS = distance between the 1st and 5th toes of the normal paw; EIT= distance between the 2nd and 4th toes of the experimental paw; NIT = distance between the 2nd and 4th toes of the normal paw.*

	NPL	EPL	NPL	EPL	NPL	EPL
	GI		GII		GIII	
1st evaluation	2.6	2.3	2.6	2.8	2.7	2.6
2nd evaluation	2.7	2.6	2.7	2.7	2.8	2.7
3rd evaluation	2.6	2.7	2.7	2.6	2.9	2.5
Mean	2.6	2.5	2.7	2.7	2.8	2.6
SD	0.06	0.21	0.06	0.10	0.10	0.10
	NTS	ETS	NTS	ETS	NTS	ETS
	GI		GII		GIII	
1st evaluation	1.8	1.8	2	2	2	1.9
2nd evaluation	1.8	1.8	2.2	1.9	2.1	2
3rd evaluation	2.1	2.1	2.1	1.9	2.3	2
Mean	1.9	1.9	2.1	1.9	2.1	2.0
SD	0.17	0.17	0.10	0.06	0.15	0.06
	NIT	EIT	NIT	EIT	NIT	EIT
	GI		GII		GIII	
1st evaluation	1.1	1.1	1.2	1.3	1.2	1.1
2nd evaluation	1.2	1.1	1.2	1.1	1.2	1.2
3rd evaluation	1.2	1.1	1.2	1.2	1.3	1.2
Mean	1.2	1.1	1.2	1.2	1.2	1.2
SD	0.06	0.00	0.00	0.10	0.06	0.06

The use of low-level laser as a therapeutic method still has contradictions and its biomodulation effect on the peripheral nerves is still unclear, since some studies present positive results[12] while others indicate that laser does not exert any influence on the peripheral nerves.[13]

Anders et al.[14] conducted a study comparing the different laser wavelengths on the facial nerve. The results confirmed all the previous studies that demonstrated that phototherapy applied transcutaneously, on a daily basis, from the first postoperative day, leads to a significant increase in the regeneration speed of the facial nerve axons in comparison to untreated animals, ascertained by the larger number of retrograde motor neurons in the spinal cord and also by the acceleration of the return of acetylcholine transferase in the facial motor nucleus.

Bagis et al.,[9] in turn, did not encounter any effect of laser therapy in peripheral nerve recovery. The rats' sciatic nerves underwent bilateral crushing, one of the sides was stimulated with 904nm laser wavelength, with different doses and evaluated with electrophysiology and morphology; however, all the analyses failed to reveal significant differences among the groups. Therefore it is hard to say whether the result of this study is strongly reliable, since it is imprecise to consider a negative result using the "untreated" contralateral side as control, if we consider that laser therapy exerts systemic effects, besides the action of nerve regeneration.

Postoperative laser therapy proved effective in the promotion of peripheral nerve regeneration in the case of complete transection and termino-terminal repair. Shamir et al.[15] observed a decrease in the degeneration process after crushing of the sciatic nerve of rats, using the HeNe laser, applied on the injured region.

The molecular basis that would justify the effectiveness of laser therapy on nerve regeneration is not yet clear. Karu[16] verified that the irradiation of isolated mitochondria induced positive alterations on cellular homeostasis. He suggested that some components of the respiratory chain (cytochromes, flavins and dehydrogenases) are able to absorb light from a particular wavelength. Thus this absorption results in an increase of ATP synthesis, affecting hydrogen levels in cells and activating ion gradient. Further studies should be implemented to seek varied wavelengths or even new repair techniques, with the purpose of elucidating the effects of laser therapy in this type of lesion.

Therapeutic ultrasound is employed for the physiotherapeutic treatment of many diseases of the musculoskeletal system, but it appears that few facts have been established with regards to its use for the treatment of peripheral nerve lesions. Nevertheless, there is some evidence in literature that this therapy may have some effect on the regeneration of injured nerves, which motivated this study. The first studies on the use of ultrasound in peripheral nerves were limited to analyzing its influence on the conduction velocity in normal human nerves, with findings that were conflicting up to a certain point. However, it was demonstrated that the peripheral nerve does not remain inert to the effects of ultrasound.

References to the use of therapeutic ultrasound irradiation as a means of stimulating the regeneration of injured peripheral nerves are relatively scarce. Hong and collaborators[17] submitted the tibial nerve of albino rats to a compression injury, followed by ultrasound irradiation, concluding that low-level ultrasound can accelerate the regeneration of peripheral nerves with compressive lesion, but adverse effects can result from the application of higher intensities. Similar effects were demonstrated in the sciatic nerve of rats submitted to a crush injury in their medial portion and irradiated with ultrasound three times a week for a month, with variation in intensity, frequency and duration.[18]

Hong et al.[17] concluded that the application of ultrasound in the intensity of 0.5 W/cm^2 contributed to accelerate the regeneration of the tibial nerve of rats after the production of a compressive lesion, but such low intensities would probably not produce the same effects in humans. Moreover, the injured nerves would have a different reaction from that of the intact nerves, and could be more sensitive to induced heat conduction, which could be the true promoter of regeneration. Anyhow, the results of these investigations were not conclusive with regards to the mechanism of action of the ultrasound, although they indicated a new application for therapeutic ultrasound.

Dyson[19] noted that ultrasound in the acute phase can stimulate the release of chemotaxic agents and cellular granulation during the cellular proliferation phase, which occurs on the 3rd post-injury day. With the exposure to the ultrasonic waves, the fibroblasts were stimulated to synthesize a larger quantity of collagen, conferring resistance to the traction of the healed soft tissues, providing stronger and more elastic scar tissue for this purpose. In this study, we evidenced a lower value in the perimeter evaluated in GIII in relation to GII and GI. This fact can explain the better recovery of the muscle system vis-à-vis the inflammatory process of the thigh region of these animals. More studies should be carried out on nerve tissue to evidence whether these muscular repercussions reproduce in nerve recovery.

The use of the gait walkway is a very common evaluation method,[4] with widespread applicability in experimental trials due to the easy execution and low cost of the method. We used the

conventional method with the wooden walkway and use of Indian ink. The normal SFI is equal to zero (0), while total dysfunction is equal to minus one hundred (- 100); hence the closer to zero, the better the function.

The values obtained in this study, with regards the SFI, demonstrate that functional loss occurred after the nerve compression injury in the three experimental groups. However, in the control group (GI), the function index decreased even further on the 7th and 14th days, while, in this period, the laser group (GII) and the ultrasound group (GIII) presented functional improvement compared to the seventh day. However, comparing GII with GIII, GII presented a greater downslide of the SFI on the 14th day.

After nerve injury, there was a tendency for adduction of the toes of the paw involved, as observed in the 1st evaluation in all the injured groups. All the animals presented heel drop during gait. In the subsequent evaluations, all the groups presented an improvement in the SFI. In the 2nd evaluation, GIII presented better evolution when compared with GII. This fact may have occurred due to the physiological properties of ultrasound in improving the inflammatory response, having analgesic function and promoting greater metabolic recovery. However, in the 3rd evaluation, GII presented better response than GIII, suggesting slower recovery, yet more prolonged in nerve recovery, when compared with GIII. The seven-day period after the injury is probably marked by these recovery events, but the use of laser therapy within 24 hours after the injury could reduce immediate functional loss, corroborating Dahlin's affirmation.[20] After the seventh postoperative day, GII presented a line of ascending tendency with regards to improvement of function.

The SFI analysis was consistent with the values obtained by the travel time analysis of these animals when evaluated. Groups GII and GIII had better responses than GI during the evaluations and GII was better than GIII when compared.

According to De Medinaceli et al.[8] by means of the crush injury, it was verified that between one and a half months and two months after the injury, functional nerve recovery reaches its plateau, although no morphometrically significant change occurs. An important fact to be mentioned involves the pre-injury functional level, noting that both groups, in this evaluation period, did not exhibit statistically significant difference, demonstrating homogeneity of the survey sample.

The results of the treatment with ultrasound were not more efficient than the laser application, but were significantly better than those of absence of treatment, observed through the weekly measurement of the SFI. In the second SFI evaluation, the values were better than the laser, but in the following evaluation, the SFI showed better recovery in the laser.

It is known that the regenerative process of a peripheral nerve is dependent mainly on the neuronal response, which concerns the cell body more than the axon. Nevertheless, the arrival of the growing axons at their target organs depends on the integrity or on the reestablishment of the neural tube. As in this study the ultrasound irradiation was applied to the lesion site, we can raise the hypothesis that the ultrasound acts only on the axon supporting tissues, contributing to allow the neural tube to reconstitute itself faster and enabling obstacles to the progression of the new axoplasma to be removed earlier. It would also make sense to think about the possibility of ultrasound irradiation stimulating the release of chemical or chemotactic mediators that would stimulate the faster production of the axoplasma. However, these are merely queries that could only be clarified with further investigations.

CONCLUSION

This study verified the benefits of low-level laser therapy and therapeutic ultrasound in nerve regeneration. It also evidenced better recovery after intervention with laser therapy when compared with the application of ultrasound in neuromotor recovery after sciatic nerve compression injury.

It is suggested that further studies should be carried out with the intention of analyzing the morphological alterations in this tissue, in order to confirm the results found with this experimental delineation.

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