ISSN 1413-3555
Rev Bras Fisioter, São Carlos, v. 12, n. 3, p. 215-21, may/june 2008
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Effect of gallium-aluminum-arsenide laser therapy (660Nm) on recovery of the sciatic nerve in rats following neurotmesis lesion and epineural anastomosis: functional analysis

Efeito da terapia com laser de arsenieto de gálio e alumínio (660Nm) sobre a recuperação do nervo ciático de ratos após lesão por neurotmese seguida de anastomose epineural: análise funcional

Reis FA[1], Belchior ACG[1], Nicolau RA[2], Fonseca TS[1], Carvalho PTC[1]

Abstract

Context: Peripheral nerve injuries result in sensory and motor losses in the innervated area and can hinder individuals' daily activities. **Objective:** The objective was to analyze the influence of applying gallium-aluminum-arsenide (GaAlAs) laser (660Nm) on the functional recovery of the sciatic nerve in rats. **Methods:** The sciatic nerve of 12 Wistar rats was subjected to injury consisting of neurotmesis and epineural anastomosis. The rats were divided into two groups: control and laser therapy. After the injury, a GaAlAs laser was used (660Nm, 4J/cm^2, 26.3mW and 0.63cm^2 beam) at three equidistant points on the injury, for 20 days. Footprint impressions were obtained from the animals before and seven, 14 and 21 days after the surgical procedure and the sciatic functional index (SFI) was calculated. **Results:** Comparison of the SFI did not show any significant difference ($p>0.05$) between the two groups. **Conclusions:** The parameters and methods used for the laser therapy did not produce any effect on the SFI over the period evaluated.

Key words: nerve regeneration; low-level laser therapy; sciatic nerve.

Resumo

Contextualização: As lesões nervosas periféricas podem comprometer atividades diárias de um indivíduo e resultam em perda da sensibilidade e motricidade do território inervado. **Objetivo:** Com o intuito de acelerar os processos regenerativos, objetivou-se analisar a influência da aplicação do laser de arsenieto de gálio e alumínio (AsGaAl, 660Nm) sobre a recuperação funcional do nervo ciático de ratos. **Materiais e métodos:** O nervo ciático de 12 ratos Wistar foi submetido à lesão por neurotmese e anastomose epineural e divididos em dois grupos: controle e laserterapia. Após a lesão, utilizou-se o laser de GaAlAs, 660Nm, 4J/cm^2, 26,3mW, feixe de 0,63cm^2, em três pontos eqüidistantes sobre a lesão, por 20 dias. As impressões das pegadas dos animais foram obtidas antes e após (sete, 14 e 21 dias pós-operatórios) o procedimento cirúrgico e calculou-se o índice funcional do ciático (IFC). **Resultados:** A comparação do IFC não resultou em diferença significante ($p>0,05$) entre os grupos. **Conclusões:** Conclui-se que os parâmetros e métodos empregados na laserterapia demonstram resultados nulos sobre o IFC no período avaliado.

Palavras-chave: regeneração nervosa; terapia a laser de baixa intensidade; nervo ciático.

Received: 17/07/2007 – **Revised:** 12/11/2007 – **Accepted:** 14/03/2008

[1] Physical Therapy Program, Universidade para o Desenvolvimento do Estado e da Região do Pantanal (Uniderp) – Campo Grande (MS), Brazil.
[2] Tissue Biomodulation Laboratory, Research and Development Institute, Universidade do Vale do Paraíba (Univap) – São José dos Campos (SP), Brazil
Correspondence to: Filipe Abdalla dos Reis, Rua Goiás, 1.709, Vila Célia, CEP 79022-355, Campo Grande (MS), Brazil, e-mail: fi_abdalla@terra.com.br

Introduction

Peripheral nerves are often exposed to injuries from traumatic origins, such as crushing impact and total transections, resulting in decreases or complete loss of sensory and motor capabilities in the corresponding area of innervation. The severity of these deficits will depend on the involvement of various structures. The impairment on the daily activities of patients with peripheral nerve injuries is a determining factor to establish the necessity of early recovery[1].

The functional recovery following peripheral nerve injury has intrigued researchers for a long time, and it has generated a great number of scientific studies about the several intervention techniques used, the phenomena involved in regeneration and the evaluation methods for the results. Although there is always some recovery in most nerve injuries, they occur slowly and, many times, in an incomplete manner[2]. It is estimated that in some countries, the incidence of traumatic injuries is greater than 500.000 of new cases annually[1], where 2.8% of the patients acquire permanent incapacities due to the length of time required for the nerve regeneration, of around two months[3]. This fact explains the elaboration of different therapies to reduce the levels of injury and incapacity.

To evaluate the levels of nerve injuries in experimental situations, the functional assessment of gait has been shown to be a reliable and reproducible method[4,5]. In 1982, De Medinacelli, Freed and Wyatt[5] proposed the use of a method of assessment named the Sciatic Functional Index (SFI) based upon measurements of the rear feet of rats. This method was used with a normal control group and in experimental groups, after the sectioning and crushing of the sciatic nerve. The experiment consisted of obtaining images of the animals' footprints, while they walked on a track specifically built for this purpose and the images were recorded and analyzed.

The use of therapeutic resources with a regenerative purpose is a common practice in Physical Therapy. For peripheral nerve injuries, the electric stimulation[6], ultrasound therapy[7] and low-intensity laser therapy (LILT)[8] have been used to accelerate the regenerative processes, aiming at the early recovery of the patients' functional status. LILT was used on the regenerative processes and the functional recovery from peripheral nerve injuries in the 1970's, and there were various reports and disagreements over the obtained results[9].

By the analyses of this study, it was found that the He-Ne laser, with an emission in the red region of the electromagnetic spectrum, is the most studied wave length in the biomodulation of biological responses in the healing process. At this very moment, new wave lengths are now being developed and researched, like lasers in the 650 to 830Nm region. It can be observed that, in many studies, the descriptions of parameters for irradiation like: dosages, average power, lengths and methods of application are widely varied, which makes the methodological understanding difficult for reproducing results and for other comparisons.

Considering the poor reproducibility studies cited in the literature and the previously observed unsatisfactory methodological presentation of the data, this study aimed to evaluate the effects of low intensity laser therapy on the repairing processes of the sciatic nerve. The investigation of an experimental model related to the analysis of functional recovery could provide relevant data as the basis of future clinical applications in the treatment of nerve injuries.

Materials and methods

Animals

Twelve male adult rats three month old were used, from the Wistar breed, with a body weight ranging from 300 to 350g, brought from the Central Bioterium of the Universidade para o Desenvolvimento do Estado e da Região do Pantanal (Uniderp), kept under controlled conditions of luminosity and temperature, with six animals per cage, and regular feeding and water *ad libitum*.

The rat was chosen as the experimental animal for its ease of acquisition, handling and also for the low operating costs. Besides these factors, the similarities to the human distribution of nerve trunks and the proper anatomic characteristics for surgery procedures facilitated the choice of this animal[1].

All experimental procedures were done according to the rules from the Brazilian College of Animal Experimentation (Cobea), and approved by the Ethics Committee of Research from the Universidade do Vale do Paraíba (Univap), under the record number L185/2005/CEP.

Animals were randomly assigned for two experimental groups, according to the procedure to be performed:

- the control group (n=6): where the animals were submitted to unilateral injury by neurotmesis of the sciatic nerve, with epineural anastomosis and without irradiation;
- the laser therapy group (n=6): with the animals submitted to unilateral injury by neurotmesis of the sciatic nerve, with epineural anastomosis and subsequent irradiation with laser on the injured area for 20 consecutive days.

Surgery procedures

After weighing all animals, each one received an administration of the pre-anesthetic butorphanol (Turbogesic,

2mg/kg) associated with acepromazine (Acepran, 1mg/kg), both in single intramuscular doses. After 15 minutes, zolazepam and tiletamine (Zoletil 50, 40mg/kg) were administered. Once anesthetized, the animal was positioned in the ventral decubitus, with its front and back paws in abduction. This was done with the alcohol-iodized anti-septic , trichotomy and incisions on the side face of the right thigh, from the height of greater trochanter to the knee. The sciatic nerve was addressed, and with the aid of a magnifying glass, about 3mm distal from its appearance, a nerve injury by complete transection of the sciatic nerve was carried out. After the injury, an epineural anastomosis of the sciatic nerve was done with three simple stitches using nylon monofilament (Mononylon 10-0, Polysuture®, Minas Gerais, Brasil). After completion of the injury, the soft tissues were sutured with simple stitches, using nylon monofilament (Mononylon 5/0, Ethicon®). Following the surgery, each animal received a single dose of Fentanyl® by intraperitoneal via (0.032mg/kg) aimed at infection prophylaxis and analgesic promotion, respectively. Over the next two consecutive days, analgesic was administered every 12 hours. The injury by complete transection was preferred in this study rather than by a crushing injury, since the first one preserves the structure that supports the nerve, increasing the axonal extension, as the neural tubes are in continuity, thus facilitating the regeneration.

Laser therapy

The gallium-aluminum arsenide (GaAlAs) diode laser from the brand KLD® (Amparo, São Paulo, Brasil), model Endophoton, with the wave length of 660Nm, power of 26.3mW, beam of light area of 0.63cm^2, was used in the continuous mode. The application was done by the transcutaneous punctual and by contact method (to reduce reflection), with an energy density of 4J/cm^2, a power density of 0.0413W/cm^2 and a duration of 96.7 seconds. The mean power of the equipment was verified prior to the trial with the aid of a power meter (2-Watt Broadband Power/Energy Meter, Model 13 PEM 001/J, Holland). Three points were irradiated over the surgical incision: one point in each extremity and another point in the centre. The laser therapy began during the first post-surgery day and was applied over the following 20 days. The animals in the control group were submitted to the same procedures, however, with the laser off.

Functional analyses

Animal footprints were obtained before and after (seven, 14 and 21 days post-surgery) the surgery procedure, through the use of millimetered paper strips that were engaged in a gait track built according to Dijkstra et al.[3] proposal. After the initial gait training of five minutes, the animals' paws were painted with nanjing ink and their footprints were recorded for the analysis of the sciatic functional index (SFI).

The collected measurements were: the distances between the second and fourth distal phalanx, between the first and fifth distal phalanx and between the proximal edge of the foot and the third distal phalanx. After these variables values were obtained, they were introduced into a mathematical equation where the results represented the percentage of the deficit in the harmed side (S=studied paw), compared to the normal side (N=normal paw). The normal function, or the absence of injury was indicated by an index of 0% while -100% represented the complete loss of function and total nerve injury (Figure 1)[10].

Data analyses

The SFI data were submitted to ANOVA analyses with post-tests of Bonferroni between the control group and the laser therapy group on the days seven, 14, and 21 of post-surgery, with significance level of 5% ($p<0.05$).

Results

The SFI of the control group was on average -35.9±48.0 of the collected data prior to the injury, -88.8±23.2 on the seventh post-surgery day; -101.9±25.9 on the 14th post-surgery

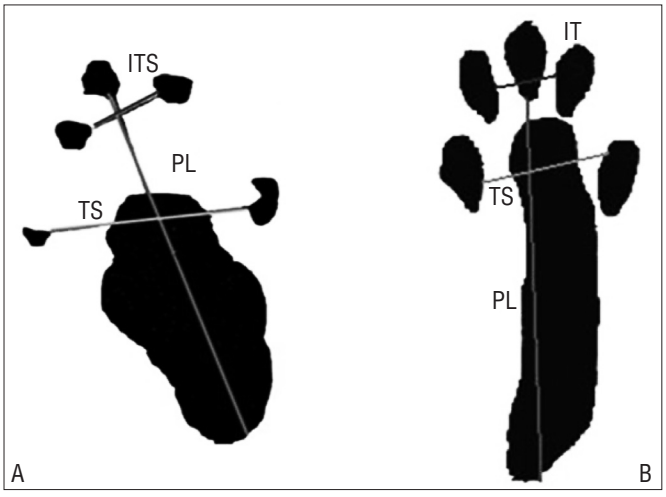

Figure 1. Representation of the values utilized in calculating the SFI after obtaining the animals' footprints, where ITS is the space between the second and fourth toes, TS is the space between the first and fifth toes and PL is the space between the heel and the third toe. Normal paw (A) is shown on the left and experimental paw (B) on the right.

day; and of -98.3±34.3 on the 21st post-surgery day. For the laser therapy group, a mean value of the collected data was obtained of -7.3±18.3 on the pre-injury moment; -98.2±18.7 on the seventh post-surgery day; -87.4±9.1 on the 14th post-surgery day; and -79.0±11.2 on the 21st post-surgery day. The ANOVAs of the control group, for the different days of the SFI collection, showed that only the pre-injury values were statistically significant ($p<0.001$) when compared to the other days (Table 1). For the laser therapy group, the pre-injury values compared to the seventh, 14th, 21st days post-surgery showed statistically significant differences ($p<0.001$). However, there were no difference between the seventh, 14th, 21st days post-surgery ($p>0.05$).

In comparison between the two experimental groups (control *versus* laser) it was noticed that there were no significant differences in the values between the four periods of evaluation ($p>0.05$) as show in Figure 2.

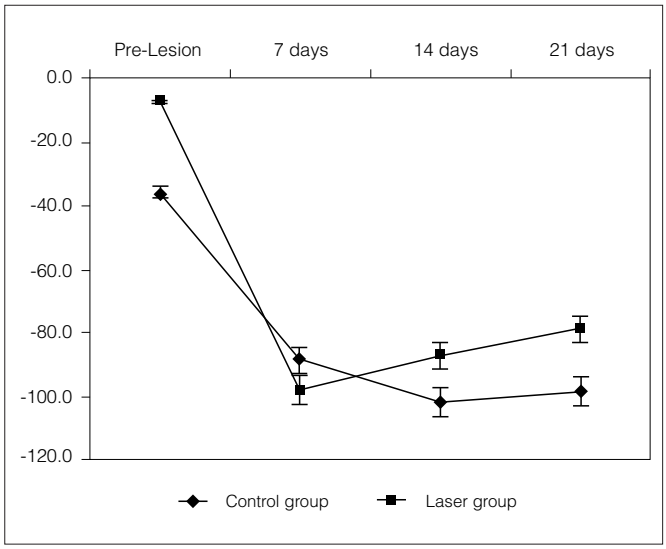

Figure 2. Graph representing means and standard deviation on the different SFI evaluation dates, for both groups.

Discussion

There are evidences, in experimental and clinical researches[11] that one of the laser's effects is to improve nerve function, to avoid wound development[12], to elevate neurons metabolism and to increase the capacity to produce myelin. Since laser therapy is not invasive, the ability to radiate injured nerves without surgical interventions is beneficial. Endo[13] claimed that the injury by neurotmesis introduces a number of variables hard to control and to standardize, however we did not find many difficulties in the present study, since the injury was standardized in all animals of both experimental groups and the surgical technique was executed by an experienced professional with specialized training. Another important fact for choosing this type of injury is because of the shortage of studies that use laser in the injury by transaction.

A variety of surgical procedures has been used in the repair of peripheral nerves, including epineural and perineural repair, autogenic grafts, vein grafts and entubulation, with or without association of neurotrophic factors[14]. The simple epineural anastomosis was the adopted technique because of its easy execution and because it has shown high biomechanical resistance to traction, according to Temple et al.[15].

The He-Ne laser (632.8Nm) in the red emission region of the electromagnetic spectrum was the most studied wavelength on the biomodulation of biological response in the repair process[16,17]. Lately, others wavelengths are being developed and researched, like the lasers that emit radiation in the bands of 650-830Nm (GaAlAs)[18,19] and that of 904Nm (GaAs)[20,21]. It was adopted the 660Nm GaAlAs laser because of its low intensity and for having a wavelength often used in clinical practice, besides that, there are no significant number of previous studies over this wave length and its effects on peripheral nerve regeneration[22]. In clinical practice, the low

Table 1. The different SFI evaluation dates, for both groups, with their means and standard deviation, respectively. Note comparative analyze between the groups, where: $^{a}x^{b}<0.05$; $^{a}x^{c}$, $^{a}x^{d}<0.01$; $^{e}x^{f}$, $^{e}x^{g}$, $^{e}x^{h}<0.001$ and other comparisons didn't demonstrate significant difference.

Rats	Sciatic functional index							
	Control				Laser			
	Pre lesion[a]	7days[b]	14days[c]	21days[d]	Pre lesion[e]	7days[f]	14days[g]	21days[h]
R1	-6.60	-79.72	-93.30	-93.30	-9.53	-125.48	-93.30	-76.73
R2	-18.38	-93.30	-124.53	-62.53	-25.88	-79.59	-75.30	-90.81
R3	-24.91	-128.91	-141.94	-135.81	-1.58	-93.30	-93.30	-83.75
R4	-133.07	-77.90	-84.94	-91.53	23.16	-116.28	-93.30	-60.13
R5	-19.48	-77.90	-73.49	-65.19	-27.18	-93.30	-93.30	-87.91
R6	-12.71	-93.30	-93.02	-143.38	-2.97	-81.17	-75.91	-74.59
Mean	-35.9	-88.8	-101.9	-98.6	-7.3	-98.2	-87.4	-79.0
SD	48.0	23.2	25.9	34.3	18.6	18.7	9.1	11.2

intensity laser therapy (LILT) employs doses of 1 to 4J/cm^2, associated to an output power between 10 and 90mW, being widely used in various musculoskeletal injuries, in addition to the algic and inflammatory processes[12]. Based on this fact the use of a density of 4J/cm^2 in this study was justified. It is important to highlight that this parameter is widely variable in the researches using laser therapy on nerve regeneration. The use of LILT as a therapeutic method still has contradictions and its biomodulator effect over the peripheral nerves is obscure yet, since some studies show positive results[18,21,22] while others indicate that the laser has no influence over the peripheral nerves regeneration[19].

The use of the gait track is a very common method of assessment[23] that has a wide applicability on experimental researches for its easy execution and low cost of the method. Some researches are trying to modernize this data collection with the use of digital cameras making it possible a more dynamic evaluation[3]. However, the purpose of this research better fits in the use of the conventional method with a track of wood and the use of ink nanjing. The values obtained in this study, related to the SFI, showed that, after the nerve injury by complete transection, there was a severe functional loss in both experimental groups on the seventh day post-surgery, however, in the control group the functional index reduced even more on the 14th and 21st day while, in the same period, the laser therapy group showed functional improvement compared to the seventh day, although the statistical analysis did not show significant results between groups.

A probable explanation for the low SFI of the irradiated group on the seventh day post-surgery was the fact that, on the first hours after the axon rupture, the cellular body starts to present alterations (cromatolisis), histologically characterized by cell ingurgitation, Nissil substance degeneration (neuron rough endoplasmic reticulum) and nucleus migration from the center to the periphery. These alterations, in spite of the neurotransmitters production, aim at increasing protein synthesis (actin and tubulin), which are related to the axon cytoskeleton regeneration, and affect intracellular transportation and growth cone movement[24]. Probably, the seven days period after injury is marked by these events, but the use of laser therapy within the 24 hours after injury, could reduce the immediate functional loss, confirming the Dahlin[25] claim. After the seventh day post-surgery, group 2 (laser therapy) presented a line of upward trend regarding to functional improvement, and this line was stabilized and upward only in group 1 (control) after the 14th day. On the 21st post-surgery day it was observed that there were no differences between the SFI of laser and control groups. Nevertheless, when the evolution of the curves on Figure 1 is analyzed, it is observed that the mean values were different, showing a probable continuity of the laser action over the sciatic nerve.

De Medinacelli[26] found that after one month and a half to two months after a crushing injury, functional recovery reaches its plateau, although there is no morfometrically significant change. An important fact to be mentioned is the functional level previous to the injury, noting that in both groups, in the evaluation period, they did not present any significant statistical difference, thus showing the uniformity of this research sample.

Although there is no statistical significance between the experimental groups, when the SFI is evaluated, it can be observed that on group 2 (laser) there were no signs of infections and points of suture dehiscence, which was observed in some animals from the control group (dehiscences). Another observed fact was that the animals from this group often used the injured limb for support during feeding, standing up on two members and even to scratch themselves, while in the control group there was dehiscence formation, making it clear the difficulty to heal the injury of adjacent tissues.

The molecular base that could justify the laser therapy effectiveness over the nerve regeneration is not clear. Karu[27] verified that the irradiation of isolated mitochondria induced positive changes over the cell homeostasis. He suggested that some components of the respiratory chain (cytochromes, flavins and dehydrogenase) are able to absorb the light from a certain wavelength. Thus, this absorption results in an increase of ATP synthesis, affecting the cell levels of hydrogen, activating the ionic gradient (sodium, potassium, calcium).

Although the functional improvement is related to the increase of the myelin sheath, other events may justify this improvement. These would include: an increase on energy transportation and calcium spread in the cytoplasm that would create an increase in the functional cellular potential[27], an acceleration on the injury repair and an increase on scarring tissue resistance[28], pro-inflammatory action, as the acceleration on tissue regeneration and the improvement on blood circulation. The increases in tissue regeneration are due to the following effects: 1) increase in the activity of leukocytes, phagocytes, and an increase of calcium in the cell cytoplasm; 2) acceleration of cell division and growth; 3) activation of protein and cytokines synthesis; and 4) relaxation of blood vessels' walls (vasodilatation) by photolysis of complexes like the NO. Consequently, these reactions produce indirect effects, such as bactericidal, regenerative and vasodilatory. Karu, Pyatibrat e Afanasyeva[29] claimed that the nitric oxide is also associated with energy production, stimulation of mitochondrial biogenesis and apoptosis,

related to analgesic effects and with an increase on microcirculation. However, new studies should be implemented, to investigate the role of different wave lengths and other parameters established by several repairing techniques, with the purpose of clarifying the laser therapy's effects on this type of injury.

Conclusion

The use of gallium-aluminum arsenide LILT (660Nm) did not culminate in positive results over functional recovery in the sciatic nerve of rats after injury by neurotmesis followed by epineural anastomosis.

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