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ORIGINAL RESEARCH

Comparison of different low-level laser therapy wavelengths in the soleus of Wistar rats after nerve injury

Comparação de diferentes comprimentos de onda do laser de baixa potência no sóleo de ratos Wistar após lesão nervosa

Comparación entre diferentes longitudes de onda de láser de baja potencia en el sóleo de ratas Wistar tras lesión nerviosa

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ABSTRACT | Skeletal muscles may be affected by peripheral nervous system injuries, leading to muscle weakness and atrophy. Several therapeutic resources may be used in the attempt to recover the functionality of muscles, such as low-level laser therapy (LLLT). This study compared the effect of LLLT of two wavelengths (660 nm and 830 nm) on morphological characteristics of muscle tissue after axonotmesis of ischiatic nerves of Wistar rats. A total of 32 Wistar rats were divided into four groups: G1 (control), G2 (injury), G3 (injury and treatment with 660 nm LLLT) and G4 (injury and treatment with 830 nm LLLT). G2, G3, and G4 animals were submitted to sciatic nerve damage and, three days after the injury, G3 and G4 were treated with LLLT of 660 nm and 830 nm, respectively. After the treatment, all animals were euthanized, and the soles muscles were collected to perform morphological analyzes of the tissue using histological slides. We verified that animals submitted to the lesion underwent morphological changes in the fiber, resulting in their atrophy. We also noticed that LLLT with a wavelength of 830 nm presented slight signs of recovery of the morphometric characteristics analyzed.
Keywords | Sciatic Nerve/injuries; Laser Therapy; Physical Therapy

RESUMO | Os músculos esqueléticos podem ser afetados por lesões do sistema nervoso periférico, levando a fraqueza e atrofia muscular. Na tentativa de recuperar a funcionalidade dos músculos, existem vários recursos terapêuticos utilizados, dentre os quais o laser de baixa potência (LBP). Este estudo comparou o efeito do LBP em dois comprimentos de onda (660 nm e 830 nm), em características morfológicas do tecido muscular após axonotmese de nervos isquiáticos de ratos Wistar. Para tanto, foram utilizados 32 ratos Wistar, divididos em quatro grupos, sendo G1 (controle), G2 (lesão), G3 (lesão e tratamento com LBP de 660 nm) e G4 (lesão e tratamento com LBP de 830 nm). Os animais de G2, G3 e G4 foram submetidos à lesão do nervo isquiático e, três dias após a lesão, G3 e G4 realizaram tratamento com LBP de

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660 nm e 830 nm, respectivamente. Após o tratamento, todos os animais foram eutanasiados e os músculos sóleos coletados para confecção das lâminas histológicas, visando a realização de análises morfológicas do tecido. Constatou-se que os animais submetidos à lesão sofreram alterações morfológicas na fibra, resultando em sua atrofia. Foi percebido também que o LBP com comprimento de onda de 830 nm apresentou ligeiros sinais de recuperação das características morfométricas analisadas.
Descritores | Nervo Isquiático/lesões; Terapia a Laser; Modalidades de Fisioterapia.

RESUMEN | Las lesiones en el sistema nervioso periférico pueden afectar los músculos esqueléticos y provocar debilidad y atrofia muscular. Para recuperar la funcionalidad de los músculos, se utilizan varios recursos terapéuticos, entre los cuales el láser de baja potencia (LBP). Este estudio comparó el efecto del LBP en dos longitudes de onda (660 nm y 830 nm) sobre las características morfológicas del tejido muscular después de la axonotmesis de los nervios ciáticos en ratas Wistar. Para ello, se utilizaron 32 ratas Wistar, divididas en cuatro grupos: G1 (control), G2 (lesión), G3 (lesión y tratamiento con LBP de 660 nm) y G4 (lesión y tratamiento con LBP de 830 nm). Los animales de G2, G3 y G4 se sometieron a lesión del nervio ciático y, tres días después de la lesión, el G3 y G4 se sometieron al tratamiento con LBP de 660 nm y 830 nm, respectivamente. Después del tratamiento, todos los animales fueron sacrificados y se recogieron los músculos sóleos para la preparación de placas histológicas, con el fin de realizar análisis morfológicos del tejido. Se encontró que los animales sometidos a lesión sufrieron cambios morfológicos en la fibra, lo que resultó en atrofia. También se observó que el LBP con la longitud de onda de 830 nm presentó leves signos de recuperación de las características morfométricas analizadas.
Palabras clave | Nervio Ciático/lesiones; Terapia por Láser; Modalidades de Fisioterapia.

INTRODUCTION

Peripheral nerves are structures often affected by several types of traumatic injuries, such as compressions and sections. As a consequence, the transmission of nerve impulses is interrupted, which causes a decrease or loss of sensitivity and motor function of the innervated area. Due to its location and extension, the sciatic nerve is predisposed to lesions that affect the muscle tissue related to its path, with fiber atrophy and structural changes that impair motor functions of lower limbs[1,2].

Therapeutic measures focused on the treatment of musculoskeletal lesions aim to relieve pain and reduce inflammatory process. Thus, several approaches are applied, among which the administration of corticosteroids or nonsteroidal anti-inflammatory drugs, therapeutic heat modalities, controlled physical activity, and low-level laser therapy (LLLT)[3].

LLLT is a noninvasive therapy that has as benefits reduction of pain and acceleration of the tissue repair process[4] by vasodilation and microvascular proliferation, increased phagocytic activity, reduced release of nitric acid and cyclooxygenase-2, increased release of growth factors, and increased collagen deposition[3,5,6]. Specifically in peripheral nerve repair, LLLT works in the increase of Schwann cells, axonal diameter and myelin sheath thickness, with decreasing their destruction [6,7], thus promoting better functionality, due to nerve recovery[8-10].

However, some conflicting results regarding LLLT exist, especially related to nerve repair, which can be explained by large dosimetric variations, such as different energies, type of radiation and wavelengths[11,12]. According to Smith[13], wavelengths within the red spectrum would be absorbed directly by cytochrome c oxidase into mitochondria, whereas near-infrared wavelengths would be absorbed by molecules present in the plasma membrane, thus producing different physiological and, consequently, therapeutic effects. Therefore, this study compared the effect of LLLT within the red (660 nm) and infrared (830 nm) fields on morphological and morphometric characteristics of muscle tissue after axonotmesis-type injury of Wistar rats sciatic nerves.

METHODOLOGY

This is an experimental and cross-sectional study, carried out at the Western Paraná State University (Unioeste) and approved by the Research Ethics Committee on the Use of Animals of the institution.

The sample group was composed of 32 ten weeks-old Wistar rats, kept in polypropylene plastic boxes

in an environment with 23±1ºC as mean temperature and a light/dark cycle of 12 hours, in addition to free access to water and feed. Then, animals were divided into four groups (n=8):

- G1: absolute control, no injury;
- G2: animals submitted exclusively to injury;
- G3: animals submitted to both injury and 660 nm laser treatment;
- G4: animals submitted to both injury and 830 nm laser treatment.

Sciatic nerve injury protocol

For the axonotmesis lesion, animals of G2, G3, and G4 were anesthetized with ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg) intraperitoneally. Following, the specimens were submitted to trichotomy and incision parallel to the fibers of the femoral biceps of the right thigh, exposing the sciatic nerve. Then, the nerve was compressed with a hemostat for 30 seconds, on the second tooth of the rack and pinion[14].

Treatment procedure

On the third day after injury, the treatment procedure was initiated. Low-level laser (Ibramed®) used was measured in relation to power, using wavelengths of 660 nm and 830 nm, respectively, for G3 and G4. The treatment was performed for two weeks, with an interval at the weekend, totaling 10 applications at the site of nerve compression. The parameters are presented in Table 1.

Table 1. Dosimetric parameters for groups that underwent low-level laser therapy

Wavelength	660 nm	830 nm
Creep	10 J/cm²	10 J/cm²
Energy	0.6 J	1.2 J
Power	30 mW	30 mW
Irradiance	0.5 W/cm²	0.25 W/cm²
Output area	0.06 cm²	0.12 cm²
Application time	20 s	40 s

Preparation of histological slides

At the end of treatment, the animals were anesthetized and euthanized by guillotine decapitation. The right soleus muscles were removed, cleaned and fixed in 10% formalin. Then, the material was diaphanized, impregnated and included in paraffin. Cross-sections of 7 µm thick were performed in a microtome and stained by hematoxylin-eosin (HE).

Analysis of histological slides

In total, 10 images were captured in the microscopic fields to count 100 fibers, using a light microscope coupled to a digital camera with 40× lens. These images were analyzed using the Image-ProPlus 6.0 program in relation to the area and the smallest diameter. Analyses of the number of muscle fibers and myocyte nuclei were also performed; the relation between the number of nuclei and the number of muscle fibers; muscle fiber density and nuclei density in an area of 31.684 µm², examining five fields for each group. Finally, morphological analysis was performed, in which muscle tissue characteristics were observed, described and presented as morphological planks, observing the shape and integrity of muscle fibers, in addition to the position of myonuclei. All analyses were performed by the same blind researcher.

Statistical Analysis

Data were analyzed by the BioEstat 5.0 program and they were presented in a table by mean and standard deviation, with comparisons between groups by unidirectional Anova, with post-LSD test. In all cases, we adopted 5% significance level.

RESULTS

The results for area and smaller diameter show that the injured groups presented significantly lower values than those of the control group, and only area presented a significant increase of the group treated with 830 nm (G4) in relation to the injury group (G2). For the number and density of fibers, all groups were significantly different, with increasing values for G1, G4, G2 and G3. Regarding the number and density of nuclei, G1 presented significant differences in relation to the others, which also occurred between G3 and G4. Finally, for the nuclei/fiber ratio, all groups differed significantly from each other, with decreasing values of G1, G4, G2 and G3 (Table 2).

Table 2. Presentation on mean and standard deviation of the values obtained for different groups (G1-G4) for the area of muscle fibers (μm²), smaller diameter (μm), number of fibers and nuclei (area of 31.684 μm²), density of fibers and nuclei, and core/fiber ratio

	G1	G2	G3	G4
Area	23,342.25±2,448.44 A	9,481.29±2,084.38 B	9,782.01±2.090.15 BD	11,922.91±2.015.67 D
< Diameter	107.50±17.53 A	64.83±8.50 B	71.96±9.21 B	65.44±7.23 B
Number of fibers	21±1.2 A	40.2±1.9 B	56.2±2.4 C	36.8±1.6 D
Fiber Density	0.66±0.04 A	1.27±0.06 B	1.77±0.07 C	1.16±0.05 D
Number of nuclei	63±3.4 A	100.8±1.6 BC	103.4±2.7 B	100±1.6 C
Nuclei Density	1.99±0.11 A	3.18±0.05 BC	3.26±0.08 B	3.16±0.05 C
Nuclei/fiber ratio	3.01±0.22 A	2.51±0.12 B	1.84±0.08 C	2.72±0.13 D

Equal letters denote statistical similarity, that is, when no similar letters are presented within the same variable, for each group, there is a significant difference. E.g.: area G1 was different from the others; G3 was similar to G2 and G4, but G2 and G4 were different from each other.

Regarding morphological analysis, it was possible to observe that the control group maintained normal characteristics, with muscle fibers in polygonal shape, intact myofibers, and peripheral nuclei (Figure 1A). The injury group (G2) exhibited several alterations, and the fibers were atrophic, with amorphous shape, disorganization of myofibers and presence of number of enlarged and globose nuclei with basophilic halo, located inside the fibers (Figure 1B). The treatment groups G3 and G4 presented morphological characteristics similar to those of the injury group (G2) (Figure 1C and 1D, respectively).

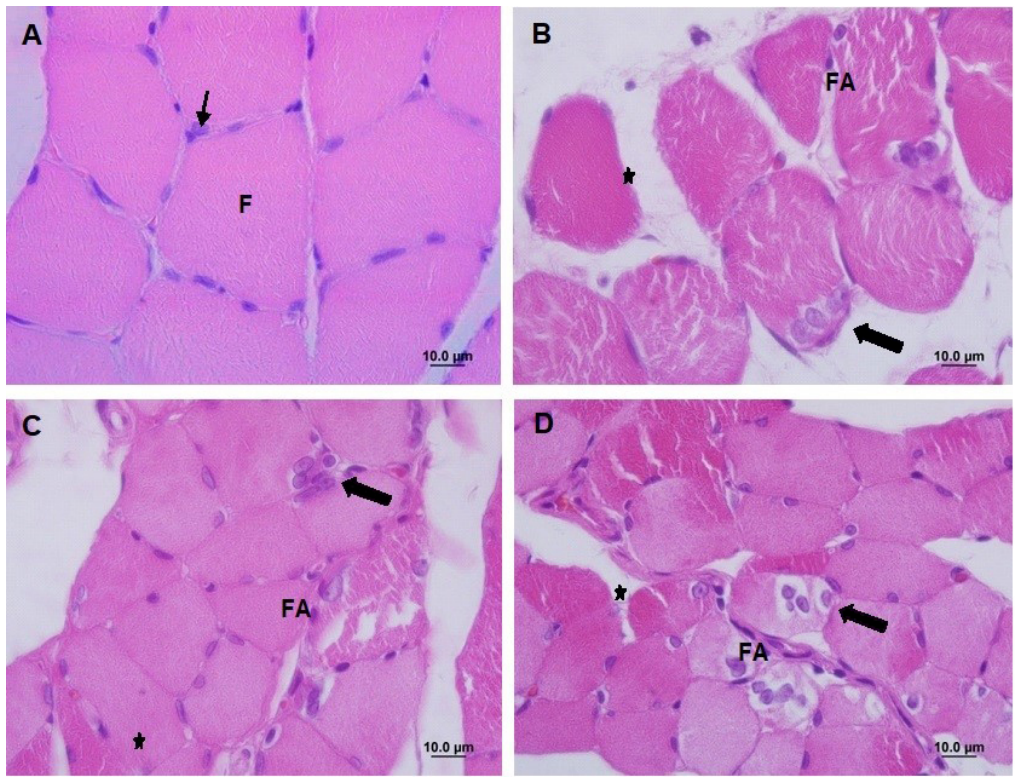

Figure 1. Morphological analysis of the soleus muscle of Wistar rats in cross-section, HE staining. A: control group (G1), with normal morphological characteristics of muscle tissue, polygonal fibers, and homogeneous myofibers (F), with peripheral nuclei (thin arrow); B: injury group (G2); C: 660 nm laser-treated injury group (G3); D: 830 nm laser-treated injury group (G4). In groups B, C and D, muscle fibers were atrophic in amorphous shape (AS), with dyeing differences, denoting myofibers disorganization (star), number of enlarged and globose nuclei located inside the fiber with basophilic halo (hollow arrow).

DISCUSSION

Recovery from peripheral nerve injury is repeatedly assessed by functional tests[8], and, as one of the main effects of this injury is precisely on skeletal muscle tissue[1], this study aimed to compare two wavelengths of LLLT in morphological characteristics of muscle tissue recovery after sciatic nerve compression, although

none of the wavelengths tested has produced this recovery, presenting muscle fibers with morphological characteristics similar to the control group without injury. Only slightly better results were observed for the group that used the 830 nm wavelength, regarding the area of muscle fiber.

It is believed that wavelengths within the red spectrum can be absorbed into cytochrome c oxidase, the terminal enzyme of the respiratory chain in mitochondria, while wavelengths within the infrared spectrum would be absorbed by proteins in the cytoplasmic membrane, thus generating similar biological effects, but with different metabolic cascades[13].

Regarding morphometric analysis, by the measurements of area and smaller diameter, it is evident that all injury groups presented atrophy of muscle fibers. Santana et al.[1,15] observed, in a similar model of nerve injury, muscle fibers characteristics of tropism, with reduction in strength and phenotypic changes. In our study, only the group using 830 nm obtained a higher result than the injury group for the area of muscle fibers, observing a small evolution. However, this result was not confirmed in the evaluation of the smaller diameter of muscle fiber. The study also presented, for the control group, a lower number of muscle fibers, myonuclei, fiber and myonuclei density, which can be explained by the hypotrophy resulting from muscle injury for the other groups and thus, by the evaluated spectrum, an increase was observed in all these variables. Note that the values observed for the low-level laser of 660 nm group were higher in number and density of fibers, indicating to worse results even when compared to the injury group. On the other hand, the 830 nm group, despite not presenting results similar to those of absolute control group, showed lower values than the other injury groups, thus suggesting advantages of this therapy.

Machado[16] reports that during the process of muscle regeneration a proliferation of satellite cells occurs, presenting consequent increase in the number of nuclei, so that the hypertrophy capacity is expanded. Each nucleus is responsible for a volume domain of muscle fiber, causing an increase in total cell volume. This explains the increase in the number of nuclei in the injured groups observed in this study, both in total values and in the density of nuclei, and the 660 nm group presented higher values than the 830 nm group, that is, the first had worse results. Notably, as a higher number of fibers were found in the injured groups, the relationship between nucleus/fiber presented lower values for the control group, which also occurred in the comparison between G4 and G3.

Based on the morphological analysis, it was possible to notice that G2, G3, and G4 underwent characteristic changes of muscle injury and that there was no return to normal muscle properties compared to the control group. The normal morphology of muscle fibers is described as multinucleated polygonal cells, with the nuclei positioned in the periphery[1,17], similar to what was found in the control group. In the nerve-damaged groups, some cells with amorphous shape and globose nuclei were found, some of them inside the sarcoplasmic membrane. Moreover, signs indicating muscle injury were found, with the presence of inflammatory infiltrate, nuclei inside the sarcoplasmic membranes, and increased cell nuclei, which can be explained by the dorsal root reflex[18].

Note that only morphological and morphometric analyses of muscle tissue were performed. Thus, possibly, the observation of other properties could show different results in relation to the treatment used. Therefore, it is suggested to conduct further studies evaluating other characteristics, such as the presence of inflammatory markers and contractile function. Finally, despite similar creep and power, the energy delivered, irradiance, applicator output area, and irradiation time were different, possibly being intervening factors in the results.

FINAL REMARKS

Therefore, sciatic nerve injury caused atrophy and morphological changes in the muscle tissue of Wistar rats. Furthermore, it seems that treatment with LLL therapy at a wavelength of 660 nm produced deleterious results in soleus, whereas 830 nm wavelength presented slight signs of recovery of the morphometric characteristics analyzed. Considering that different wavelengths were compared, with similar power and energy density, other dosimetric factors were also different.

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