The low-level laser on acute myositis in rats[1]

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ABSTRACT

PURPOSE: To analyze the effects of the low-level laser therapy in the acute myositis induced in rats.

METHODS: Fifteen male Wistar rats (*Rattus norvegicus*) were randomly distributed into three categories: control group (C), induced Myositis without treament (MI) and treated induced myositis (MT), for the experimental period of seven days. The induction of the acute myositis was done with 1% acetic acid by intramuscular route. The rats belonging to the experimental group had daily treatment through the GaAs (gallium arsenide) laser, with 904,0 nm length, and 45 mW peak power, with 3J/cm^2, applied for 5 minutes on the animals' right posterior limb.

RESULTS: In the MT group there was a statistic significant decrease in the number of inflammatory cells, related to the MI group ($p<0.05$), increase in the fibroblastic proliferation, when compared to groups C and MI related to MT group ($p<0.01$) and statistic significant edema regression ($p=0.0400$) in the MT group.

CONCLUSION: The low-level laser therapy was efficient in the reduction of the inflammatory process, increase of the fibroblastic proliferation and the reduction of the edema.

Key words: Inflammation. Myositis. Laser Therapy, Low-Level. Rats.

Introduction

The skeletal muscle is a tissue subject to injuries regarding the daily and sportive activities requested from them. The inflammation is an important process for the regeneration of the injured tissue, but the continuity of it is always considered undesirable. The structural and functional morbidity happens in the forms of atrophy, contractures, pain and the increase in the likelihood of recurrence[1].

The inflammation in muscles fibers can occur due to the high impact activities together with high intensity functional load, which may cause injuries in the tissue structure. For this reason ruptures may happen in the cytoskeleton structures, leading to morphological and biochemical changes in the muscle fibers, as a result of the fibers injuries and the present inflammatory process[2].

The low-level laser therapy presents bio-stimulatory effects and has been used to repair distinctive tissues, among them the muscular tissue. It has been seen that its use leads to the increase in the number of mitosis and in the development of the epithelial cells, providing the increase of the circulation and the synthesis of collagen by the fibroblast in the injured site[3-7].

The laser therapy acts in the regeneration process of the skeletal muscle after the injury. It helps the increase of the mitochondrial respiration and the ATP synthesis, decreases the inflammatory response and stimulates the new formation of blood vessels. This resource also induces the regulatory protein synthesis in the skeletal muscle's satellite cells, because of the activation of the cell cycle since the injured skeletal cells are not replaced by new ones. In skeletal muscle system additional satellite nuclei are obtained, which multiply, and therefore merge into the injured fibers[8].

The goal of the present study was to analyze the effects of the low-level laser therapy in the induced acute myositis in rats.

Methods

The study was approved by the Ethic Committee of the Faculdade Diferencial Integrada (FACID), Teresina-PI, Brazil (Nº 055/14) and it was done according to the Law Nº 11.794 Arouca/2008.

Fifteen male Wistar rats (*Rattus norvegicus*), with the age between 30 to 40 days and weighing 200-250 grams, were used. The animals were kept in individual cages in the vivarium of the institution and fed with ration (Labina™) and water *ad libitum* under a cycle of light/darkness.

The animals were randomly distributed into three equal groups, containing five rats in each group, using the experimental period of seven days, distributed as follow: Group I: control group (C), group II: Induced Myositis without treatment (MI) and group III: Induced Myositis treated with GaAs Laser, with 904.0 nm length (MT) (n=5 per group).

Initially the animals were weighed and then according to their weight were anesthesied with 10% ketamine hydrochloride, given 0.1 mL for each 100 grams of bodyweight, associated with the same dosage of 2% xylaxine through intramuscularly route[12] in the left posterior paw of the animal.

The rats belonging to the experimental group were treated with the low-level laser therapy after the induction of the myositis. The laser used was the GaAs laser, Physiolux Dual Laser (Bioset), with the wave length of 904.0 nm and 45 mW peak power, that sends out the wave in a pulsate way, which is characterized by the application of the laser over the surface, for a determined time the irradiation with the laser was done in the 3J/cm^2 dosage applied in the posterior right limb during five minutes (Chart 1). The gastrocnemius muscle was submitted to the laser application with contact between the activated point in 90 degrees and the muscular layer in six different and equidistant locations, involving also the peripheral area, which allowed the whole muscle to receive the treatment equally.

CHART 1 – Laser application parameters.

Laser As Ga (Physiolux Dual) Bioset	
Wave length	904,0 nm
Potency	45 mW
Application method	Ponctual
Dosage	3J/cm ^{2}
Application time	5 minutes

The length measurement of the right posterior limb was done through the use of a digital caliper from the King Tools® brand, having as limit the tibio-femoral and tibio-calcaneus joints. Half of this measurement was marked with an anatomic marker, serving as a standard for the antero-posterior diameter (APD) and the latero-lateral diameter (LLD) measurement in the rats' posterior right limbs. The measurement of the APD and the LLD was done during the experimental period, with the use of digital caliper. These diameters were used for the evaluation of the developing and evolution of the edema in the animals´ right posterior limb in the following days after the injection of the acetic acid[14].

The animals were sacrificed with an overdose of Sodium Thiopental anesthetic (50mg/kg). After the euthanasia a biopsy

of the gastrocnemius muscle was done, afterwards the surgery tools of interest were singly coded, fixed in 10% formaldehyde, and subjected to histological processing routine, with histological cuts in the digital axis of 5 µm of thickness and then dyed with haematoxylin – eosin.

The histological analysis was done with the Labomed iVu3100 microscope attached to a photograph camera NA030. The images were acquired with the PixelPro™ programme under the magnification of x40. The ImageJ® programme in function of the cell counter was used to determine the number of inflammatory cells and fibroblasts in the experimental groups (Figure 1).

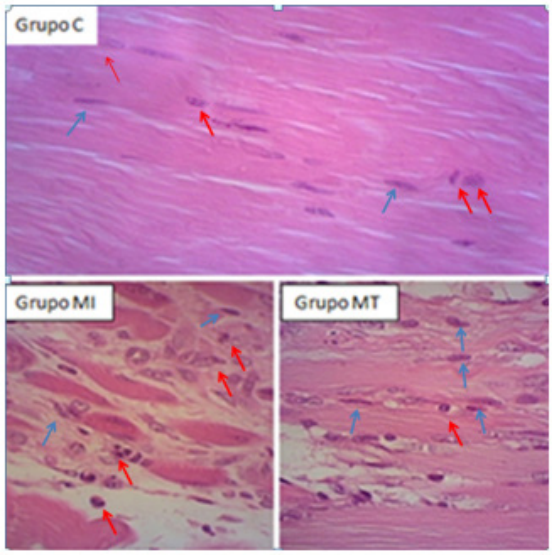

FIGURE 1 – Illustrative pictures of the histological analysis colored with Haematoxylin-eosin in the groups C, MI and MT. Red arrows (inflammatory cells) and the blue arrows (fibroblasts**).**

The data was organized in the Microsoft Office Excel 2010 spreadsheets. Afterwards, the groups were subjected to statistics comparisons analyses, One-way ANOVA following Tukey Test, with a confidence interval in 95% and significance in $p<0.05$. For this purpose the data was transferred to the statistic program Graph Pad Prim 5.0.

Results

According to Figure 2, where the number of inflammatory cells found in each experimental group was analysed, it was observed that there was a significant statistic difference among the Control group (C) and the Induced Myositis (MI) with ($p<0.05$), which proves the presence of the myosites, induced with acetic acid. In the MT group there was a significant difference, regarding the number of inflammatory cells, related to the MI group with ($p<0.05$). This leads to the thought that the GaAs Laser was efficient in reducing the muscle inflammatory process, by the reduction of inflammatory cells quantity.

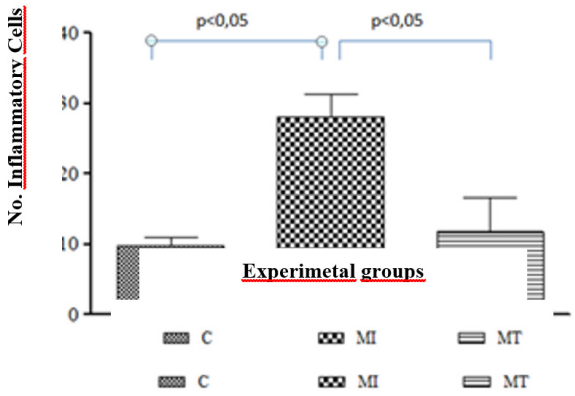

FIGURE 2 – Number of inflammatory cells (20.000 µm$^{2)}$ found in the experimental groups studied in the 7 day treatment with the GaAs Laser.

In the evaluation of the fibroblasts number, represented in Figure 3, it was observed that when comparing groups C and MI to group MT the results were statistically significant with ($p<0.01$). This demonstrates that there was an increase in the number of fibroblasts in the experimental groups studied, giving evidence that there was an increase in the proliferation of fibroblasts favoring the repairing process in the tissue.

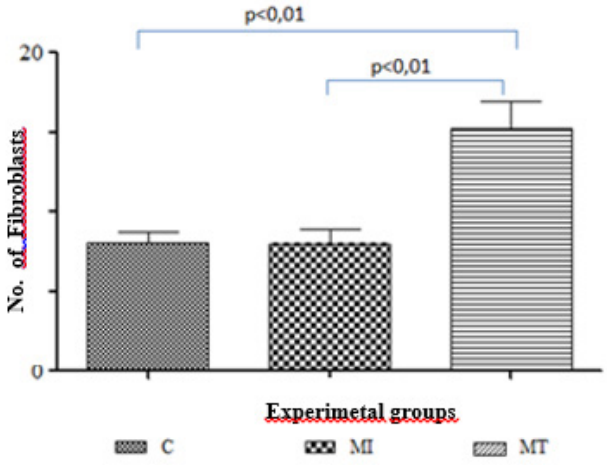

FIGURE 3 – Number of fibroblasts (20.000µm^2) found in the experimental groups studied in the 7 day treatment with the GaAs Laser.

The results of this study show that in the MT group it was observed that after the 2nd day of GaAS laser treatment there was a regression in the LLD, statistically significant ($p=0.0095$), reaching the same value as the group C in the 4th day of laser

treatment. In the MI group there was a decrease in LLD, (Figure 4). The data suggests that the laser decreased the edema present in the groups.

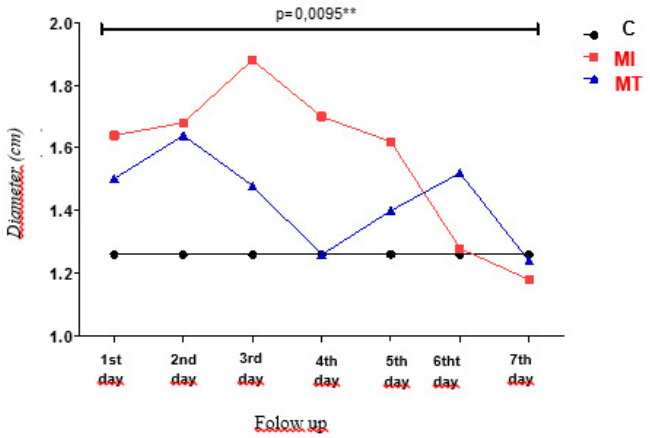

FIGURE 4 - Evaluation of the latero-lateral diameter of the groups in the experiment treatment of the acute myositis induced in rats. P for the One-way ANOVA test with a 95% interval of trust and the significance in $p<0.05$.

In Figure 5 it can be noted that in the experimental period there was a significant modification statistically ($p=0.0072$) in the APD in the MI and MT groups, showing that there was a decrease in this diameter in the groups studied.

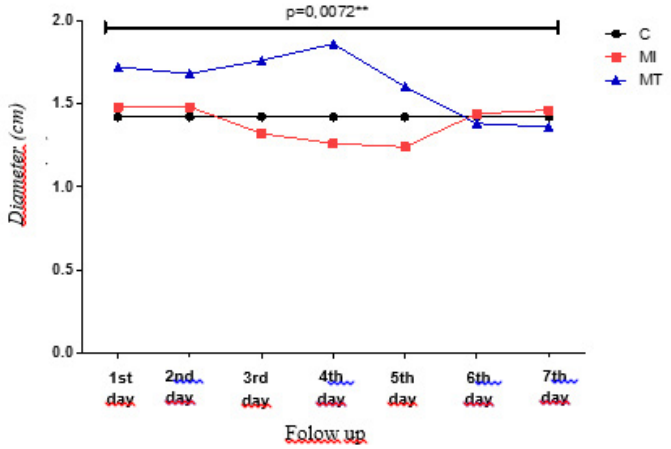

FIGURE 5 - Evaluation in the antero-posterior diameter of the groups in the experiment treatment of the acute myositis induced in rats. P for the One-way ANOVA test with a 95% interval of trust and the significance in $*p<0.05$.

Discussion

Studies suggest that the effects of the low-level laser therapy promote the reduction of the inflammatory process in some distinctive body tissues. However, even though numerous studies confirm the laser biomodular capacity, the parameters and the ideal protocols for its application haven´t been established yet[3,11-14]. That reinforces the necessity of new studies with the objective to accomplish a better suitability of the parameters and protocols for each application.

In the present study the use of GaAs laser was chosen, with a wave width of 904nm because of the equipment's capacity to provide deeper insertion in the tissue, by being gently absorbed by the hemoglobin and water. It is also well indicated for muscles, bones and tendons treatment, because of its photosensitivity properties over collagen and bone cells[14].

According to the analysis of the results of the present study it was seen an improvement in the inflammatory process of the groups investigated. Similar results were found in another research, where the goal was to analyze the muscular regeneration in elderly rats after concussion under a laser irradiation of 830nm, with a $4J/cm^2$dosage. It was seen that after 21 days of treatment, there was a significant reduction in inflammation area, highlighting that with the parameters used, the laser showed efficiency in the muscular regeneration in elderly rats through its anti-inflammatory effect[15].

Based on the results obtained in the present research, a satisfactory effect that the low level laser exercises on the musculoskeletical tissue inflammatory process was observed, which can be proved through the reduction in the number of inflammatory cells after the treatment. The edema, present in the myositis process, negatively interferes in the injured muscle, making it possible for it to lead to a function deficiency for which it is responsible, possibly being therefore, an important inflammatory signal to be treated during the therapy. The laser demonstrated benefits in the reduction of the edema due to the inflammatory process in the distinct tissues.

Significant results of the laser under the improvement of the inflammatory process in the muscular tissue were also found in another study, which had the goal to show the effectiveness of the laser application of 960 nm, $2J/cm^2$ in the injury recovery process provoked by impact in the gastrocnemius muscles of rats. The irradiation was done for three days, with two daily applications, in the injured area. The groups' histological analyses showed the presence of blood vessels and hematomas in the repairing process of the group subjected to the laser irradiation. It leads to the belief that the laser therapy positively affects the regeneration of the muscular injury, in accordance with the present research[16].

The laser seems to be efficient in the repair process of distinct tissues. Studies prove its repairing potential over the scar tissue healing of the sciatic nerve on rats subjected to crushing,

with a laser irradiation of 830nm with a 4 J/cm² dosage for seven and 14 days. With results analyses it was possible to observe the significant statics difference compared to the groups under the treatment which showed a bigger quantity of fibroblasts and fewer quantity of inflammatory infiltrate[13].

Studies prove the beneficial effects of the laser over the regeneration of the muscular tissue. A comparative analyses was done between two dosages of the Helium-Neon laser (HeNE) (5 and 10J/cm²). The irradiations were done on the tibial muscle of injured rats. It was observed that in the experimental groups subjected to the laser treatment there was an increase of the mitochondrial activity, the fibroblasts and macrophages activities and the angiogenesis of animals' anterior tibial muscle, favoring the repairing of it[17].

Another study also showed a qualitative improvement in the groups irradiated with the laser, benefiting the tissue regeneration. It was done with the objective to evaluate the action of the 670 nm laser in the process of repairing muscular tissue in the rats after gastrocnemius muscle contusion. The treatment was done in the 1st to the 4th day with a dose 3J/cm², using a punctual technique. In the groups irradiated with the laser a tissue better organized structurally was observed, with a greater quantity of integrated muscular cells, less fragmented fibers and less noticeable signs of intercellular edema in relation to the non-irradiated muscles[18].

A study was done with the intention of analyzing the effects of the low-level laser in the manifestation of collagen after muscular lesion. The animals were submitted to a daily irradiation of 05 J/cm² with Gallium/Aluminium/Arsenide (GaAlAs) and GaAs lasers and, in different sacrifice periods (seven and 14 days). The data pointed that the dosage of 05 J/cm² of the laser GaAs 904 nm promoted a better disposition of collagen fibers after 14 day treatment, suggesting that therapy would be effective in synthesis of collagen[3], which suggests that the GaAs is effective in the regeneration of the muscular tissue.

The data that refers to the reduction of edema agrees with another study, in which the goal was to evaluate the use low-level laser over the pain and edema of calcaneus tendon of rats subjected to the experimental trauma, where the irradiation was applied with the 670 nm laser and the dosages of 2J/cm², 4J/cm² e 8J/cm². The results evidenced that there was a formation of edema in all of the groups after the lesion and for the treated groups there was a reduction of edema, showing that the low-level laser treatment brought a reduction of edema in the animals with tendon trauma[19].

The low level laser therapy is a painless method, non invasive, at a low cost and practically without side effects. Its bio-modular action in the tissues allows a faster tissue recovery; with effective pain relief action.

Conclusions

The low-lever laser therapy seems to be effective in inflammatory process. The tissual repair in rats was achieved through the reduction of the inflammatory cells with the increase of the fibroblasts and the significant reduction of edema.

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