ORIGINAL ARTICLE

BIOMECHANICAL AND HISTOLOGICAL ANALYSIS OF THE GASTROCNEMIUS IN RATS SUBJECTED TO MUSCLE INJURY AND TREATMENT WITH LOW-LEVEL LASER THERAPY

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ABSTRACT

Objective: To mechanically and histologically evaluate the application of low-level laser therapy to the reparative process on lesions caused by impact on the gastrocnemius muscles of rats. Methods: 45 female Wistar rats were divided into three groups (n=15/group): C (control, no lesion), ML (muscle lesion) and ML-L (muscle lesion and laser therapy). The experimental muscle lesion was produced by letting a 250 g load drop from a height of 30 cm, directly onto the muscle. The animals in the ML-L group were subjected to application of 960 nm laser, 2 J/cm^2, on the lesion site, for three days, twice a day. Mechanical tests were performed on an Emic® universal testing machine. Results: The mean values for the maximum force were: 35.70 (± 2.69) N in group C, 31.77 (± 2.59) N in group ML and 34.36 (± 3.63) N in group ML-L, with a statistically significant difference between groups C and ML ($p < 0.05$). The mean values for relative stiffness were: 3.75 (± 0.98) N/mm in group C, 3.84 (± 0.32) N/mm in group ML and 4.43 (± 0.68) N/mm in group ML-L, with no statistically significant differences ($p>0.05$). Histological analysis showed the presence of blood vessels in group ML-L and hematomas during the repair process. Conclusion: Laser therapy had a positive effect on the regeneration process of the muscle injury.

Keywords – *Laser therapy, low-level; Rats; Regeneration*

INTRODUCTION

Among muscle injuries, 90% consist of bruises or sprains[1]. Bruises are caused by rapid direct trauma in which compressive force is applied to the muscle tissue[2], whereas sprains occur when muscle stretching exceeds the elastic capacity. Whatever the trauma mechanism is, pain is present. This interferes with locomotion and with performance in sports activities and daily life[3-6]. The high variability of such injuries has placed limits on conducting clinical studies[7] and on determining the most effective treatment[8].

With the aim of minimizing these variables, previous studies have produced muscle injuries invasively, through muscle compression using forceps on animals[9,10].

Other authors have used experimental models of muscle injuries in animals by means of noninvasive techniques, such as spring-loaded hammer systems[11] and weight release techniques, from predetermined heights[6,12-14].

In 1975, Järvinen and Sorvari[15] developed an experimental model for muscle injury using a spring-loaded hammer. These authors produced injuries in the sural triceps and, two days later, they observed that the changes produced were uniform. Other authors[16] used the technique of weight release (700 g) from a height of 12.5 cm and observed that it was important to determine the force, tissue deformation and energy transfer to the muscle. On another occasion[12] when the weight release technique was used, a height of 102 cm and mass of 171 g were used, and a load cell was coupled to the system to enable calculations on the energy absorbed by the muscle tissue. Nonetheless, despite studies of this

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nature, determination of the ideal mass and height for causing this type of lesion is still a matter for debate.

Just like the experimental techniques for causing muscle injuries, the treatments for them also vary[12]. Immobilization and remobilization techniques[15], cryotherapy[17] and the RICE principle (rest, ice, compression and elevation), non-steroidal anti-inflammatory drugs[18] or corticoids[19], therapeutic ultrasound[11], laser therapy[20-22] and growth factors[23] have been used with the aim of promoting faster repair of muscle injuries. Despite these efforts, no conclusions have been reached regarding the best treatment for muscle injuries[12].

Thus, the aim of the present study was to experimentally evaluate the effects from applying therapeutic laser for repairing muscle tissue that had been subjected to bruising injury, using mechanical and histological test methods.

MATERIALS AND METHODS

The design for this investigation was approved by the Ethics Committee for Animal Experimentation of the Ribeirão Preto School of Medicine: Cetea/FMRP protocol no. 069/2006.

Forty-five adult rats of Wistar lineage were used, with a mean body weight of 300 g. These animals were divided into three experimental groups (n = 15/group): group C (controls; no injury), group ML (muscle injury caused by crushing; kept without treatment) and group ML-L (muscle injury caused by crushing; treatment using AsGa laser at 960 nm).

To produce the muscle injury, the anesthetics xylazine and ketamine (0.1 mg/100 g) were firstly administered, and the posterior region of the animals' legs, around the gastrocnemius muscle, was shaved. The method used to crush the muscle was the weight release technique, from a preestablished standardized height that had been used in other studies[6,12-14]. The animals were positioned under a lead sphere weighing 250 g that was released from a height of 30 cm (Figure 1).

The animals in the ML-L group were subjected to AsGa laser application immediately after receiving the muscle injury, and then twice a day for three days. To apply the laser, the animals were kept under manual containment, without previous anesthesia. A dose of 2 J/cm^2 was used, and the duration of application was calculated automatically by the apparatus.

At the end of the experimental period, the animals were sacrificed by means of an overdose of chloral hydrate. The gastrocnemius muscles were harvested in such a way as to preserve the origin and insertion, and were destined for mechanical analyses (n = 10/group) and histological analyses (n = 5/group). The mechanical tests were performed on a universal testing machine (Emic®), model DL 10000, at the Bioengineering Laboratory of Ribeirão Preto School of Medicine, USP. The load application velocity was 10 mm/min, with preloading of 5N and an accommodation time of 30 s (Figure 2).

The parameters used for evaluating and comparing the data were the maximum force and relative stiffness.

For the histological preparation, the muscles were kept in a receptacle containing formol for seven days after dissection. After this period, the muscles were removed from the formol and sectioned transversally using a surgical scalpel, in the central region of the muscle belly, from the most external face of the muscle to the most internal face, thereby forming small blocks of 1 cm^2.

These blocks were then immersed in an ascending series of alcohols, at concentrations of 70, 80, 90% and 100%, for a 24-hour period. They were then immersed in xylol and then three times in a bath of molten paraffin,

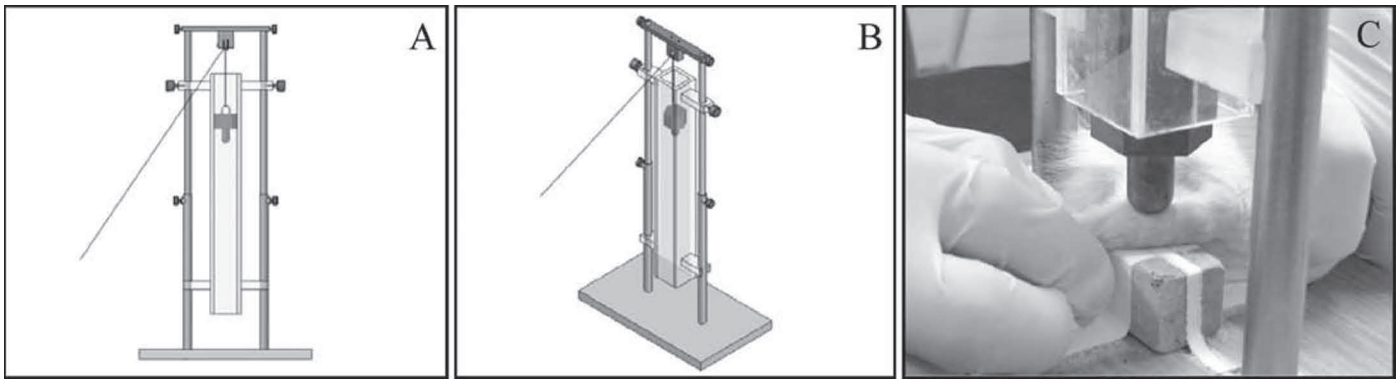

Figure 1 – Schematic drawing of the equipment used to produce the experimental crushing injury in the muscles of small-sized animals (A and B). Positioning of the animal to make the muscle injury (C).

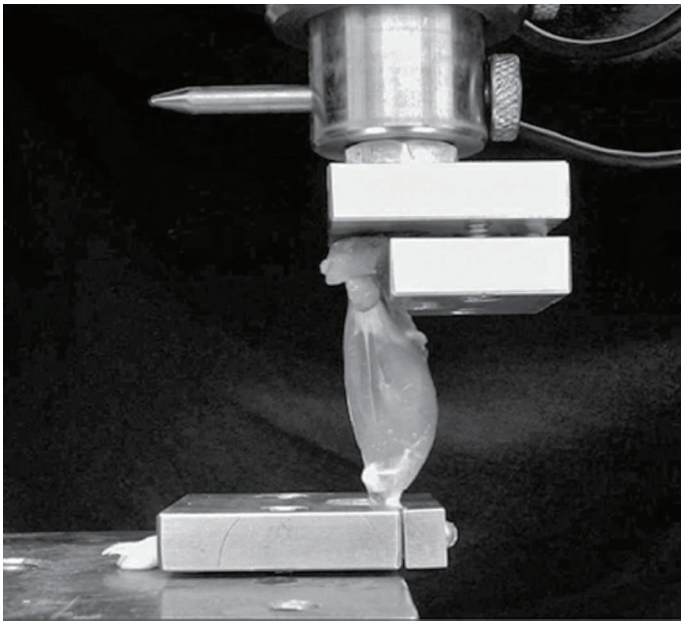

Figure 2 – Mounting and coupling of the gastrocnemius muscle to the accessories of the universal testing machine, ready to perform mechanical traction tests

in order to embed the material in paraffin blocks for subsequent sectioning using a microtome.

The sections were made transversally to the muscle fiber, with a thickness of 6 μm, obtained using glass blades in an MT 6000 XF ultramicrotome (RMC Inc.), thereby forming semi-thin tissue slides. After this process, the specimens were hydrated and deparaffinized in a hot chamber. The slides were then mounted, stained with hematoxylin-eosin (H&E) and fixed with Permot® resin for subsequent analysis under an optical microscope at a defined magnification, and using an image analyzer, in order to observe the hematoma area and other visible alterations.

The semi-thin transverse sections from the segments were observed under an *Axioimager* M1 photomicroscope (Carl Zeiss), using objective magnifications of 20, 40 and/or 100 times, with immersion in oil. The images of the muscles were digitized by means of a high-resolution camera (*Axiocam* MR; Carl Zeiss) and transferred to a microcomputer (IBM/PC – AT Pentium® III) in which they were stored on the hard disc for subsequent analysis. The areas of hematoma were identified by visual inspection and were counted.

The results were subjected to the normality test, to ascertain whether the behavior of the data was parametric or not, using multifactorial analysis of variance (multifactorial Anova). To make comparisons between the groups, Tukey's post-test was used. For all the analyses, the significance level used was 5% ($p \leq 0.05$).

RESULTS

Mechanical analysis

Thirty gastrocnemius muscles were tests, and the values were expressed as means and standard deviations for each of the properties of the three groups analyzed.

Maximum force

The means for the maximum force values were: (35.70 ± 2.69) N in group C, (31.77 ± 2.59) N in group ML and (34.36 ± 3.63) N in group ML-L. A statistically significant difference was observed between the groups C and ML ($p < 0.05$) (Figure 3).

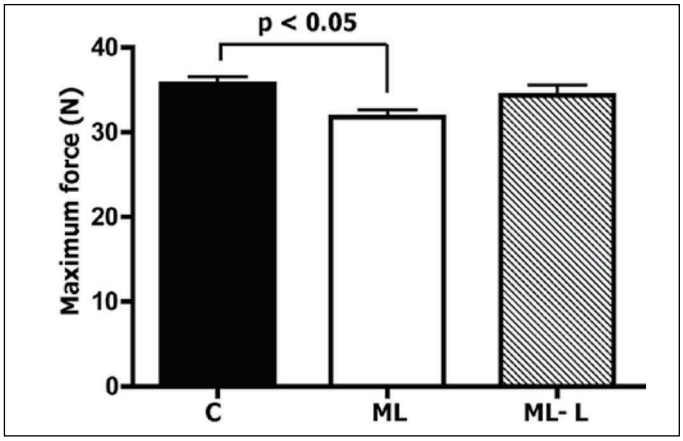

Figure 3 – Mean values for maximum force

Relative stiffness

The means for the relative stiffness values were: (3.75 ± 0.98) N/mm in group C, (3.84 ± 0.32) N/mm in group ML and (4.43 ± 0.68) N/mm in group ML-L. There was no statistically significant difference between the groups ($p > 0.05$) (Figure 4).

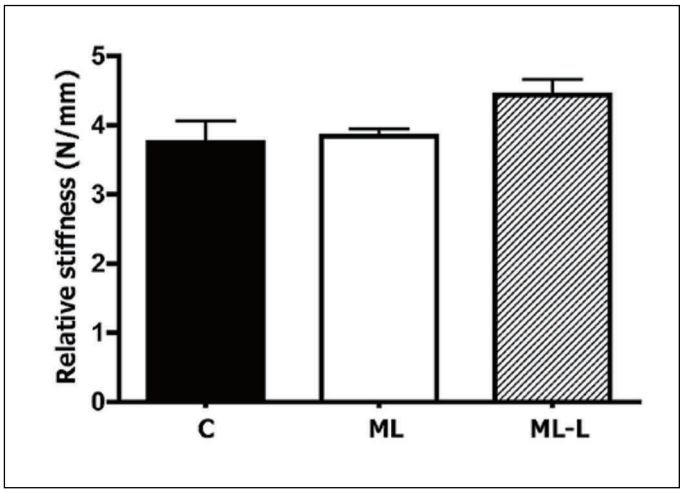

Figure 4 – Mean values for relative stiffness

Histological analysis

On the third day after the injury, the histological sections from the muscles in groups ML and ML-L showed the presence of hematomas, cell infiltrate and tissue disorganization. The magnification of 40 times made it possible to observe neovascularization in group ML-L and hematoma undergoing a repair process (Figure 5).

DISCUSSION

Muscle injuries are one of the most common forms of injury resulting from sports[2,19]. They can be caused by bruises, sprains or laceration[2], although bruises and sprains predominate[1].

Despite the high incidence of muscle injuries caused by bruises, the studies in this field present limitations because of the high variability of such injuries[7]. Thus, experimental models with standardized production of muscle injuries are very important in attempting to elucidate the rehabilitation methods for muscle bruising. There are several techniques for producing muscle injuries of bruise type, among which some are invasive, using a forceps inserted through the skin incision[10] and others are noninvasive, using spring-loaded hammer systems[11] or weight release systems[6,12-14,16]. After establishing and standardizing the muscle injury methodology, techniques and procedures for speeding up muscle recovery can be administered and compared. The techniques are varied, including physical agents such as ice[24]; medications[8]; mechanical agents such as immobilization[25]; and electrotherapy resources, such as low-level ultrasound[11] and laser[20-22]. However, it remains unclear which technique is best, and there are divergent results in the literature.

Oliveira *et al*[20] studied the effect of AsGa laser for regenerating the anterior tibial muscle of rats that were subjected to treatment for five consecutive days at the respective doses of 3 J/cm^2 and 10 J/cm^2. The authors concluded that the treated groups presented morphological factors resembling those of the control group, but an increase in muscle mass was observed in the group irradiated with 10 J/cm^2.

In another study more recently[22], the investigators observed that application of AsGa laser for seven days, at doses of 5 J/cm^2 and 10 J/cm^2, provided

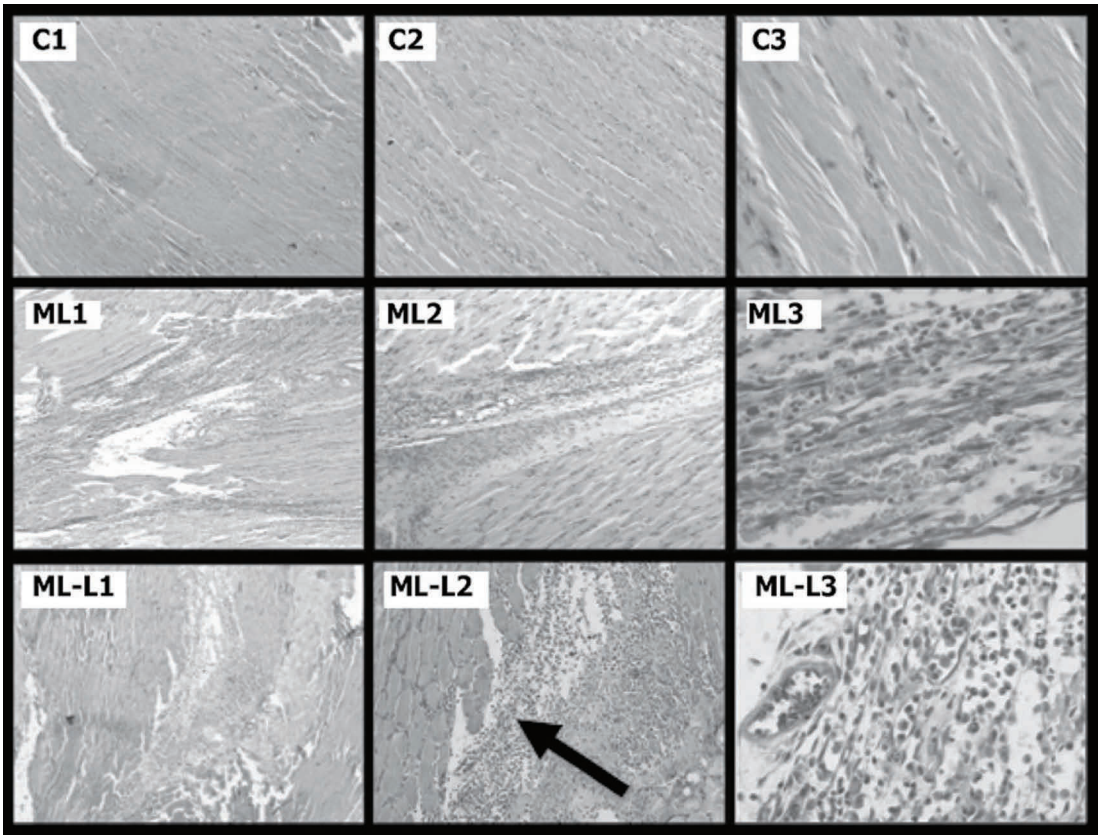

Figure 5 – Muscle fibers of the gastrocnemius muscle of the control group (C), muscle injury group (ML) and muscle injury with laser therapy group (ML-L). The arrow points to a blood vessel that formed in the ML-L group. Images captured with objectives of magnification 5X (C1, ML1 and ML-L1), 10X (C2, ML2 and ML-L2) and 40X (C3, ML3 and ML-L3).

increases in mitochondrial activity, fibroblast and macrophage action and angiogenesis of the anterior tibial muscle of rodents.

In our study, a difference in maximum force was observed between the control group and the group of animals subjected to muscle injury without treatment, such that loss of mechanical resistance in the injured muscle was noted. Comparing the control group with the group of animals subjected to muscle injury and laser therapy, no difference was observed. In other words, application of the laser therapy promoted mechanical recovery of the previously injured muscle. With regard to stiffness, no statistical difference was observed between the groups, but the group of animals subjected to laser treatment presented greater stiffness than did the other groups.

Histological analysis was performed on the three groups after three days (C, ML and ML-L), for comparison purposes.

A morphological difference was observed among the muscles of the rats subjected to muscle injury. The histological findings showed evident hematomas in the groups subjected to muscle injury (ML and ML-L). It should be noted that the group of animals treated with laser (ML-L) presented the beginnings of tissue repair, with less cell disorganization and lesion area. In addition, vessel formation could be observed in this group, thus showing the angiogenesis capacity of laser therapy. No statistical analysis was performed on the histological results, since quantitative evaluation of the slides was not our objective.

CONCLUSION

The results from this study allow the conclusion that laser therapy positively influences the regeneration process on muscle injuries.

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