Research Article

Pharmacokinetic and Pharmacodynamics of Sodium Diclofenac (Topical and IM) Associated with Laser Photobiomodulation on Skeletal Muscle Strain in Rats

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Purpose. The practice of physical activities is considered a primary factor for the maintenance of good health status. However, exhaustive or unusual physical activities can lead to muscle injuries. Several treatments are used to recover muscle injuries; however, systemic NSAIDs often result in serious adverse events. In this study, we aimed to investigate the association of laser therapy (LLLT) and topical diclofenac, evaluating the kinetics of the drug and its pharmacodynamic effect in stretching-induced muscle injury in rats.

Methods. Male Wistar rats weighing 200 g were randomized and divided into groups of 6 animals. Plasma concentrations of diclofenac were quantified by mass spectrometry at different times (15 min to 24 hours) in all animals. The laser energy used was 3 Joules (830 nm; 100 mW, 30s). Treated groups received diclofenac at the dose of 1 mg/kg IM or topically applied with or without laser therapy. The electric stimulation was used to study the functional status of the muscles. Results. After topical administration of diclofenac, the peak plasma concentration (t max) occurred for 30 minutes in the irradiated group and 4 hours in the nonirradiated group. The AUC (0-24 hs) was 442 (ng/h/mL-1) in the nonirradiated group and 712 (ng/h/mL-1) in the irradiated group. Conclusion. LLLT was effective to provide a significant improvement in functional patterns. Taken together, our results demonstrate the synergistic effect between LLLT and topical diclofenac in muscle injury induced by stretching in rats.

1. Introduction

Unusual and exhaustive physical exercise may lead to structural, ultrastructural, and biochemical changes of the skeletal muscle [1]. Such changes have characteristics of an acute disease called Exercise Myopathy (ME). There is evidence that the great abundance of signs and symptoms that are associated with ME is dependent on the type of contraction (eccentric vs. concentric), intensity, duration [2, 3], and individual factors, such as age and their level of physical conditioning.

Muscle injuries account for about 30% of sports injuries, of which more than 90% are caused by force or excessive stretching of the muscle [4]. The most common exercises that
can induce injuries are those with high eccentric components, such as resisted exercises, jumping exercises, plyometrics, and intermittent races with rapid change of direction [5].

There are several evidences already described in the literature regarding indirect methods of measuring muscle injury, especially in humans, including decreases of maximum voluntary isometric force [6–8], increases in muscle discomfort [9–11], significant increases in cytokines concentrations (IL-1β and IL-6), and lactate dehydrogenase and glutamic oxalacetic transaminase [12]. Levels of these mediators are usually elevated when acute muscle injury occurs.

Different types of pharmacological and nonpharmacological therapies have been used with the aim of preventing or at least mitigating the deleterious effects of exercise-related injuries. Among these, we can cite (a) anti-inflammatory drugs, (b) nutritional supplements, (c) cryotherapy, (d) massage therapy, (e) electrotherapy, and (f) low-level laser therapy.

Diclofenac is part of the nonsteroidal anti-inflammatory category and is a weak organic acid (pKa = 4.00). It is known that its main action is the ability to decrease the activity of the isoforms of the cyclooxygenase enzyme and consequent inhibition of prostaglandin synthesis [13, 14].

When orally used, diclofenac may present important adverse effects such as gastrointestinal bleeding, gastric ulcer, renal and cardiac complications, among others [13, 15]. However, in topical formulations, these adverse effects are practically not found, and their efficacy is as possible as good as oral or IM for skeletal muscle lesions [13, 16].

According to Miyatake et al. [16], the efficacy of oral nonsteroidal anti-inflammatory drugs (NSAIDs) in muscle and synovial tissues is reasonably established. In their study, they compared the plasma concentration between diclofenac given orally and topically applied, using recommended dosages in clinical prescriptions. The authors concluded that topical application is an effective method.

The efficacy of diclofenac absorption applied topically depends on the ease of transposing the different layers of the skin [17], the low molecular weight (<500 Daltons), and both hydrophilic and lipophilic characteristics to cross the stratum corneum.

1.1. Laser Photobiomodulation. Previous clinical trials have confirmed the fact that has been reported countless times. According to Samoiova et al. [18], the low-level laser therapy (LLLT) was able to increase in 32% after 2 minutes of irradiation, and in 45% after 20 minutes, the blood flow in the cutaneous microcirculation. This effect was blocked by the local application of nitric oxide synthesis inhibitor (LNMMA). Several other articles also report this increase in local microcirculation due to vasodilation in the irradiated tissue [19–23]. It seems that laser light produces vascular relaxation with elevation of cGMP [24].

In vivo experiments demonstrated a significant induction of iNOS after laser irradiation in the iliac artery of rabbits [25], and according to the results found, if laser irradiation elevates cGMP levels, a significant local vasodilation would probably occur, thus facilitating the transport of nutrients to the irradiated region as well as the removal of metabolic substances that would impair muscle contraction.

In view of the anti-inflammatory effects of low-level laser associated with its vasodilatory effects on microcirculation, we believe that laser radiation can influence or even potentiate the absorption of diclofenac applied topically. In addition, AINE’s and laser therapy may act synergistically, potentiating the anti-inflammatory effects of each one with reduced adverse effects when compared to systemic NSAIDs. In this context, the purposes of the present study were to investigate the effect of laser photobiomodulation on the pharmacokinetic of sodium diclofenac topically and intramuscularly applied and to compare the effects of both treatments on stretch-induced muscle lesion in rats.

2. Material and Methods

2.1. Animals. A total of 252 female Wistar rats from the Biotery of the University of São Paulo (USP) weighing around 200 g were used. The animals were kept under standard conditions of temperature (22-24°C), relative humidity (40-60%), light-dark cycle for 12 hours with water, and ad libitum. Rats were randomized and divided into groups of 6 animals. Our experimental protocols were approved by the Ethics Committee on Animal Experimentation of the Institute of Biomedical Sciences of the University of São Paulo (ICB_USP) n° 30 fls. page 87. For a better understanding of the experimental procedures, please see the graphical flow chart in Figures 1 and 2.

2.2. Pharmacokinetics Analysis

2.2.1. Experimental Groups for Determination of Concentrations of Diclofenac in Rat Plasma. The following experimental groups were used for the determination of plasma concentrations of diclofenac in noninjured animals: (a) healthy - healthy animals, (b) diclofenac IM, (c) laser + diclofenac IM, and (d) topical diclofenac laser + topical diclofenac;

Plasma concentrations of diclofenac were quantified by mass spectrometry at different times (0, 15, 30 min, 1, 2, 4, 6, 12, and 24 hours) in all treated and untreated animals. The laser energy used was 3 Joules. Treated groups received diclofenac at the dose of 1 mg/kg (milligram per kilogram) IM and topically applied.

2.2.2. Determination of Diclofenac Concentrations in Plasma. The determinations of the concentrations of diclofenac in rat plasma were carried out in the Cartesius Analytical Unit, Laboratory of Prof. Dr. Gilberto De Nucci in the Department of Pharmacology of the IC/USP, according to the Analytical Protocol for the Validation of METGRU Method 08-07 - Determination of Diclofenac in human plasma by LC-MS/MS.

2.2.3. Protocol for Collection of Plasma Concentration Samples. The blood samples from the animals were collected via retro orbital at different times (mentioned above). At each predetermined time of collection, about 500 μL of blood was withdrawn with capillary and placed in a clean
heparinized tube. Samples were centrifuged for 10 minutes at 4,000 RPM (Beckman GPR Centrifuge, São Paulo, Brazil). Plasma was separated into plastic tubes with a screw cap and sealing ring identified and stored in a freezer at -20°C for further analysis.

Samples from each animal were analyzed on the same day, thus avoiding interassay variations. Plasma concentrations of diclofenac were measured by reverse phase high-performance liquid chromatography (HPLC), and the peaks were monitored by mass spectrometry (MS/MS) detectors.

2.2.4. Extraction of Plasma Samples. The procedures described were applied not only to unknown samples but also to standard curve extraction and quality controls. During the run of extraction of the unknown samples, these occurred under yellow light, the following experimental protocol was followed. An appropriate number of 12 × 120 mm disposable glass test tubes were placed in a grid. The tubes were numbered according to the assay protocol; the plasma (100 μL) of the animal samples were added to each tube; in each tube, 50 μL of internal standard (2 μg/mL Naproxen solution) was added using a calibrated autopipette, and the sample was homogenized (vortexed) for approximately 10 s; 25 μL of formic acid was added; the sample was homogenized (vortex) for 10 seconds; 4 ml of ethyl ether/hexane (80/20; v/v) was added to all tubes, and extraction was carried out by homogenization (vortex) for 40 s; the upper organic phase was then transferred to another set of clean glass tubes and evaporated under N₂ flow at 45°C; the dried residues were dissolved with 1 mL acetonitrile/water (50/50, v/v) with 1 mM acetic acid and homogenized for 20 seconds (vortex), and then the solutions were transferred to PCR plates using automatic pipettes with disposable plastic tips; the PCR plates were capped, and the plates were placed in the self-injector racks. Diclofenac and the internal standard Naproxen were extracted from plasma and analyzed by combining LC-MS/MS with turbospray negative and MRM detection mode. In order to conduct the tests, the quality of the reference standards used in the preparation of the solutions was evaluated. As authenticity of the reference standards, the origin and batch number of the analytical standards were recorded.

2.3. Pharmacodynamics Analysis. The pharmacodynamics analysis consists in the study of the biochemical and physiologic effects of drugs, i.e., how a drug affects an organism. Here, we investigate the effects of topical and IM diclofenac alone or associated with laser irradiation.

2.3.1. Passive Stretching Muscle Injury Model. For the tibialis muscle elongation protocol in this work, the stretching protocol was performed, as briefly described below [26].

The animals were anesthetized with ketamine: xylazine (80 : 16 mg/kg, König, Avellaneda, Argentina) intraperitoneally (i.p) before being submitted to the passive stretching protocol of the anterior tibial muscle. After weighing the animal, it was placed in dorsal decubitus on cork attached to the stretching system. The right hind limb was firmly fastened with a line, which passed through a pulley and attached itself to a pissette with a volume of water corresponding to 150% of the animal’s body. This line was fixed on the back of the animal’s paw, performing a plantar flexion, stretching the anterior tibial muscle of the right hind paw of the animal. The
protocol was performed only once and the animal received the traction for 20 minutes, resting for 3 minutes, and a second traction for 20 minutes.

2.3.2. Electromyographic Study. The animal was anesthetized with ketamine and xylazine (100 mg/kg and 20 mg/kg, respectively, IM) and fixed on a surgical table. The animal was then subjected to a cut in the skin near the metatarsal plantar region. Subsequently, a vulsion was performed, separating the tibial muscle from the subcutaneous tissue together with the skin; for this procedure, we enter the small cut already done with scissors closed on the muscle, returning in the caudal direction with the open scissors, exposing all the muscle of the animal. Then, with the aid of a scalpel, the tendon was separated from its insertion and tied to a thread. The muscle fascia was removed, making it easier to isolate the muscle. After the section, the muscle was pulled in the opposite direction to its insertion through the wire, so as to be isolated from the tibia. Throughout the procedure of stimulation of the anterior tibial muscle, it was kept hydrated with saline solution (0.09%). In the insertion region, near the metatarsal plantar region, the muscle through the tendon was connected to an isometric transducer (Ugo Basile®; Vareze, Italy) and the sciatic nerve to a bipolar electrode.

The muscle was subjected to a constant tension of 10 g. The preparation was indirectly stimulated by pulses of 6–7 V, 0.2 Hz, and 2 ms. of duration. Muscle contractions in response to indirect stimuli were recorded on the UGO BASILE® GEMINI 7070 physiographer via the isometric transducer. In order to induce tetanic contraction, the frequency was raised to 60 Hz. Muscle fatigue was characterized by the inability to maintain muscle contraction, with the amplitude decaying by 50% of the maximum recorded, to avoid tissue death, resulting from tetanic contraction. For each group, tetanic contractions were performed every 10 minutes in the period of 30 minutes, making a total of 3 contractions for each animal in each group.

From the records, we analyzed (a) the intensity of the contraction force (amplitude) in grams, (b) the time required for the contraction to drop to one-half of maximal (50% of muscle fatigue) in seconds, and (c) the area under the time curve X intensity.

2.3.3. Laser Irradiation. A Thera-lase type laser (DMC®) was used, operating at the wavelength of 830 nm in a continuous mode, at the total energy dose of 3 Joules, time of irradiation of 30 seconds, spot size of 0.028 cm², and 100 mW of power. Laser irradiation was performed in the contact mode, at only one point in the middle region of the anterior tibial muscle of the rats, after induction of muscle injury.

2.3.4. Statistical Analysis. The results were expressed as means ± SEM and submitted to the unpaired Student t-test or analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons. Values of \( P < 0.05 \) were considered statistically significant.

3. Results

3.1. Pharmacokinetics Analysis

3.1.1. Quantification of the Time Concentration Curve of Diclofenac, Applied Topically with and without Low-Power Laser Irradiation. Figure 3(a) shows the time concentration curve of diclofenac applied topically with and without low-intensity laser irradiation and quantification of plasma concentrations at different times (0, 15, 30 min, 1, 2, 4, 6, and 12 hours).

After topical administration of diclofenac, the peak plasma concentration (t max) occurred for 30 minutes in the irradiated group and 4 hours in the nonirradiated group, thus presenting a statistically significant difference for \( p < 0.05 \). The AUC (0-24hs) was 442 (ng h mL⁻¹) in the nonirradiated group and 712 (ng h mL⁻¹) in the irradiated group, where it also presented a statistically significant difference for \( p < 0.05 \).

Figure 3(b) shows the time concentration curve of diclofenac applied intramuscularly with and without low-intensity laser irradiation and the quantification of plasma concentrations at different times (0, 15, 30 min, 1, 2, 4, 6, 12, and 24 hours).

Following intramuscular administration of diclofenac, the peak plasma concentration (t max) occurred for 15 minutes in both groups. The mean plasma concentration (w/w) was 755 (ng h mL⁻¹) in the nonirradiated group and 899 (ng h mL⁻¹) in the irradiated group, showing a statistically significant difference for \( p < 0.05 \). The mean AUC (0-24hs) was 757 (ng h mL⁻¹) in the nonirradiated group and 1082 (ng h mL⁻¹) in the irradiated group, where it also presented a statistically significant difference for \( p < 0.05 \).

3.2. Pharmacodynamics Analysis

3.2.1. Effect of Tetanic Contraction on Indirect Stimulation in Rat Tibial Muscle, Postinjury, and Treatment with Low-Power Laser Therapy and Diclofenac Topic (DT) 3 and 6 Hours after the Lesion Protocol. Figure 4(a) shows the intensity of the muscle contraction force by electrical stimulation during three tetanus in a 3-hour protocol: DT, laser + DT, and laser. A muscle contraction force gain was observed in all 3 tetanus in the group treated with laser + DT (86.04 ± 3.57, 84.98 ± 3.58, and 84.78 ± 3.07) when compared to the lesion group (69.28 ± 5.75, 68.50 ± 4.43, and 67.02 ± 4.81) with \( p < 0.05 \) and the DT group (70.83 ± 4.71, 69.40 ± 5.45, and 68.30 ± 5.68) with \( p < 0.05 \).

Figure 4(b) shows the intensity of the muscle contraction force by electrical stimulation during three tetanus in a 6-hour protocol: DT, laser + DT, and laser. A muscle contraction force gain was observed in all 3 tetanus in the group treated with laser + DT (92.90 ± 3.46, 91.58 ± 3.34, and 89.88 ± 2.51) when compared to the lesion group (70.48 ± 4.14, 70.25 ± 4.82, and 70.08 ± 4.28) with \( p < 0.05 \) and the DT group (76.59 ± 3.97, 74.14 ± 6.96, and 72.67 ± 6.91) with \( p < 0.05 \). In the group treated with laser (85.78 ± 5.01, 82.98 ± 4.48, and 81.79 ± 4.02), a strength gain of muscle contraction was observed in all 3 tetanus when compared to the DT group.
Figure 5(a) shows the intensity of the muscle contraction force by electrical stimulation during three tetanus in a 3-hour protocol: D.IM, laser + D.IM, and laser. It was observed in all 3 tetanus a muscle contraction force gain in the group treated with laser + D.IM ($85.07 \pm 3.30$, $84.31 \pm 3.95$, and $84.02 \pm 3.75$) compared to the lesion group ($69.28 \pm 5.75$, $68.50 \pm 4.43$, and $67.02 \pm 4.81$) with $^*p < 0.05$ and the D.IM group. ($72.55 \pm 4.08$, $71.59 \pm 5.97$, and $69.97 \pm 5.68$) with $^*p < 0.05$.

Figure 5(b) shows the intensity of the muscle contraction force by electrical stimulation during three tetanus in a 6-hour protocol: D.IM, laser + D.IM, and laser. It was observed in all 3 tetanus a muscle contraction force gain in the group treated with laser + D.IM ($93.71 \pm 3.45$, $91.89 \pm 3.27$, and $90.69 \pm 2.49$) when compared to the lesion group ($70.48 \pm 4.14$, $70.25 \pm 4.82$, and $70.08 \pm 4.28$) with $^*p < 0.05$ and to the D.IM group. ($77.98 \pm 3.98$, $71.07 \pm 6.95$, and $70.76 \pm 6.92$) with $^*p < 0.05$. In the group treated with laser ($85.78 \pm 5.01$, $82.98 \pm 4.48$, and $81.79 \pm 4.02$), there was a gain of muscle contraction force in all 3 tetanus when compared to the lesion group and only in the second tetanus when compared to the D.IM group.

4. Area under the Fatigue Curve

Figure 6(a) shows a significant increase in all 3 contractions of the area under the muscle fatigue curve, after tetanic contraction and the ratio of muscle contraction intensity with time, so that this tension decreased by 50% of the maximum amplitude in a protocol of 3 hours when compared to the laser + DT groups ($71.98 \pm 5.33$, $73.67 \pm 5.28$, and $72.89 \pm 5.46$) and to the lesion group ($53.74 \pm 5.39$, $54.95 \pm 5.94$, and $53.12 \pm 5.63$) with $^*p < 0.05$.

Figure 6(b) demonstrates a significant increase in all 3 tetanus of the area under the muscle fatigue curve, after tetanic contraction and the ratio of muscle contraction intensity over time, so that this voltage decays 50% of the maximum amplitude in a protocol of 6 hours when
compared to the laser + DT groups (88.27 ± 5.89, 89.97 ± 5.27, and 88.63 ± 5.46) and to the lesion group (62.19 ± 6.12, 60.49 ± 5.28, and 61.96 ± 5.72) with p = 0.05 and the DT group (68.97 ± 5.65, 67.43 ± 5.44, and 66.09 ± 5.62) with +p < 0.05.

Figure 7(a) shows a significant increase in all 3 tetanus of the area under the muscle fatigue curve, after tetanic contraction and the ratio of the muscular contraction intensity with time, so that this tension decreased by 50% of the maximum amplitude in a protocol of 3 hours when compared to laser + D.IM groups (90.79 ± 5.65, 92.14 ± 5.73, and 90.20 ± 5.48) and the laser group (88.27 ± 5.89, 89.97 ± 5.27, and 88.63 ± 5.46) to the injury group (62.19 ± 6.12, 60.49 ± 5.28, and 61.96 ± 5.72) with 0.05 and the D.IM group (69.83 ± 5.38, 65.15 ± 5.72, and 65.98 ± 5.90) with +p < 0.05.

5. Discussion

In this work, the effects of low-level laser therapy operating in the infrared spectral region on the absorption and pharmacokinetics of NSAIDs (diclofenac topical and IM) and their performances separately on skeletal muscle injury were studied, using the experimental model of controlled muscle strain in the tibial muscle of rats.

The present experimental model of injury was characterized by Ramos et al. [26]. In his work, the dose of 3 J proved to be the most effective for this protocol. It was also
demonstrated that the peak of the lesion in most of the analyses was reached 6 hours after the stretching, such as Evans blue plasma extravasation, COX-2 gene expression, TNF-α, C-reactive protein, and the walking track test.

Muscle injuries often lead to a long time of immobilization and wide use of anti-inflammatory drugs, which can lead, beside the side effects, to an increase in the numbers of collagen fibers as well as a decrease in the amount of muscle fibers. Such effects contribute to the changes in the biomechanical properties of the muscle [27].

Low-level laser therapy (LLLT) represents an alternative for the treatment of musculoskeletal injuries and may have inhibitory or stimulatory effects, depending on the parameters used in the treatment. In this sense, it is common to use the term photobiomodulation to cite its effects on biological tissues. The clinical objectives of the use of low-level laser therapy in muscle injury situations are aimed at reducing the adverse effects of anti-inflammatory drug use, reducing immobilization time, and inhibiting or even reducing inflammatory process and enhance tissue repair, restoring the functional characteristics of the tissue.

As previously mentioned, the use of NSAIDs after muscle injury can lead to important adverse effects such as gastrointestinal bleeding, gastric ulcer, renal and cardiac complications, among others. Therefore, the use of topical NSAIDs has been shown also to be an interesting alternative, which, through several studies, demonstrated a good efficacy when compared to NSAIDs used by IM and oral routes, with the advantage of having few adverse effects [13, 28].

Currently, the dermatological area has been studying ways to improve the transport or transdermal absorption of some substances or drugs. To do this, a variety of media such as chemical agents, immersion, microneedles, and ultrasound is being used. However for some drugs, none of them can successfully overcome the stratum corneum (SC) layer [29, 30].

Figure 5: Panel (a) shows the intensity of muscle contraction force induced by indirect electrical stimulation every 10 minutes, in a total of 3 tetanic contractions in healthy, lesion, and intramuscular- (IM-) treated animals, 03 hours after stretching. Panel (b) shows the intensity of muscle contraction force induced by indirect electrical stimulation every 10 minutes, in a total of 3 tetanic contractions in healthy animals, lesion, and treated, 06 hours after stretching. The data represent the mean ± SEM, n = 6 (ANOVA, followed by the Student-Newman-Keuls test, 1st, 2nd, and 3rd tetany *p < 0.05 vs. lesion and 1st, 2nd, and 3rd tetany †p < 0.05 vs. DT).
Several types of laser have been tested with the objective of studying the facilitation of absorption and penetration of drugs through SC in a more effective way and without causing tissue damages such as CO2, Nd:YAG, IPL (intense pulsed light), and diodes [29, 30]. However, such studies do not assess the plasma bioavailability of the drugs used.

In few studies, high-power laser application demonstrated a great capacity of improvement in the penetration of drugs and substances through the SC, without causing damage to the skin, demonstrated through analyzes such as optical coherence tomography, VIS-NIR fiber spectrometer, spectrophotometer, and spectral scanner [29–32]. This improvement in the permeability attributed to the laser is still not fully understood.

The low-intensity laser has as its characteristics the increase of the local microcirculation and the vasodilatation and recruitment of the collateral vascularization. These characteristics are widely accepted and demonstrated in studies with muscle tissues, healing, skin flaps, etc. [19–23, 33], which could further aid in the absorption function of drugs or other substances.

The bioavailability results of the present study corroborate the studies cited above. Thus, a statistically significant difference was observed in the group irradiated with laser 5 minutes before receiving diclofenac applied topically. This group had on average a t max of 30 minutes and as mean AUC (0-12 hs) the value of 712 (ng h mL⁻¹), whereas the groups that received only topical diclofenac were evidenced

![Figure 6: (a) Relation of muscle contraction intensity over time so that this force decays 50% of maximum amplitude, in a total of 3 tetanic contractions in healthy, lesion, and topical diclofenac-treated animals, 03 hours after stretching. The data represent the mean ± SEM, n = 6 (ANOVA, followed by the Student-Newman-Keuls test, 1st, 2nd, and 3rd tetany *p < 0.05 vs. INJURY). (b) Relation of muscle contraction intensity over time so that this force decays 50% of maximum amplitude, in a total of 3 tetanic contractions in healthy animals, lesion, and topical diclofenac-treated animals, 06 hours after stretching. The data represent the mean ± SEM, n = 6 (ANOVA, followed by the Student-Newman-Keuls test, 1st, 2nd, and 3rd tetany *p < 0.05 vs. LESION and + p < 0.05 DT).](image-url)
averages of 3 hours and 442 ng h mL\(^{-1}\), respectively. In the group where diclofenac IM was applied, statistically significant results were found for AUC (0-24 hs) (ng h mL\(^{-1}\)) and C (max) (ng h mL\(^{-1}\)), 1082 and 899, respectively, with \(p < 0.05\), for the groups irradiated with laser 5 minutes before the application of the drug.

This response of the anti-inflammatory drug preceded by laser irradiation could be acting to a more rapid resolution of the inflammatory process, triggered after the occurrence of the stretch lesion, as well as better repair of the injured tissue.

In the present study, we evaluated the reduction of muscle fatigue in tibial muscle of rats after tetanic contraction by indirect electrical stimuli after stretching muscle injury, in groups treated with low-level laser therapy and with diclofenac, both applied topically and intramuscularly.

There are some definitions for muscle fatigue, and one of them says that fatigue is the inability to maintain muscle contraction force over a period of time. This disability was found in our study. The data found in our study corroborate previous results, such as those demonstrated in a study conducted by Ramos et al. [26] in our laboratory.

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As we can observe in Figures 2 and 3, the association of laser therapy and diclofenac (both topical and IM) significantly preserved the peak force of tibial muscle after 03 but especially 06 hours of the lesion. Possibly, the application of two distinguished therapies produced a synergistic effect, inhibiting the inflammatory amplification phase after muscle damage. Interestingly, topical anti-inflammatory diclofenac plus laser irradiation presented a significant enhancement of absorption and the best effects on muscle force preservation.
Concerning on muscle fatigue, we previously demonstrated that the normal behavior of nontreated muscles is a decrease of peak force and AUC in a sequence of contractions. Here, we observed that laser therapy is still the best treatment to delay the time to fatigue. Rats in the lesion group showed a decrease in fatigue resistance in all protocols, while in the laser-treated group prior to the application of topical and intramuscular diclofenac demonstrated an increase in this parameter.

According to the theory of blood flow regulation, vasoactive metabolites are released from the muscle fiber in proportion to the diffusion activities, and this metabolic vasodilatation increases the supply of oxygen and nutrients in response to the tissue demand [34, 35].

Kipshidze et al. [24] have demonstrated in their studies that visible ultraviolet light produces vascular relaxation with elevation of cGMP. The absorption of light by vascular tissue, through induction of NO, is speculated to elevate cGMP. Vasodilatation would be triggered by the hypotensive effect of both phosphodiesterase inhibitors and circulating cGMP nitrovasodilators.

In vivo experiments in laboratories demonstrated a significant induction of iNOS after laser irradiation in the iliac artery of rabbits [25], and according to our results, if laser irradiation elevates cGMP levels, a significant local vasodilatation would probably occur, thus facilitating the transport of nutrients to the irradiated region as well as the removal of metabolic substances that would impair muscle contraction. Therefore, we can assume that a vasodilatation or an increase in the local microcirculation caused by laser irradiation could be acting on the muscle cells, increasing the time for the tetanus muscle to decrease its maximum contraction intensity until reaching its half (50%).

When analyzing the area on the fatigue curve recorded in the electrophysiograph, it was possible to observe a very similar picture to that described above. Muscle fatigue presents as characteristics mainly a decrease of muscle strength and impaired motor control, with subsequent muscular pain. The presence of muscle injury also causes a decrease in performance and a reduction in the capacity to generate force.

The lesion of the skeletal muscle has as a characteristic the immediate loss of force production. One of the causes of this loss of force would be the rupture or loss of the force-generating structures, such as actin and myosin [36].

One of the mechanisms that triggers muscle injury is the excessive influx of calcium from the interstitium into the muscle fiber, resulting in elevated levels of intracellular calcium, which in turn is generated by damage to the sarcoplasm or the sarcoplasmic reticulum of the fiber. The process leads to the loss of homeostasis and stimulation to the calcium-dependent proteolysis, thus provoking a tissue degeneration [37]. In the intracellular medium, excess calcium causes the mitochondria to accumulate this ion, which inhibits cellular respiration and energy production, compromising the cell’s ability to actively remove calcium from within. Calcium overload then precipitates an autogenic phase where an increase in the action of proteases and phospholipases results in the degradation of myofibrils and the cell membrane, resulting in a compromise in glyco gen synthesis when the muscle is injured [37].

The irradiated animals increased the fatigue resistance in the three tetanus in the groups treated with laser + DT, and also in the group treated with laser alone (6 hours), demonstrating an increase in fatigue resistance superior to the groups treated with diclofenac only.

Recent studies by our group have demonstrated that the preirradiation of low-power laser athletes, both in the red and infrared wavelength range, was able to increase resistance to muscle fatigue and to accelerate the fall in the concentrations of plasma lactic acid and CK [39–41].

Skeletal muscle has a considerable ability to regenerate muscle fiber, which is limited by the satellite cell population, revascularization, and local reinnervation. All this process is slow and usually incomplete, with a great scar formation, fibrous consistency, and little elastic and may alter muscle strength and extensibility [26]. Both the extent of the lesion and the treatment are important, because as mentioned above, muscle regeneration ends up being a complex process, causing morphological changes of the regenerated fibers, which can affect postinjury muscle performance [26].

According to Amaral et al. [42], direct laser radiation, used in the first days after the partial excision of the rat gastrocnemius muscle, promoted the regenerative process and the muscular maturation of the injured region. In his research, he used different doses, demonstrating that only the dose of 2.6 J of the He-Ne laser promoted significant changes, such as increased muscle fiber area and increased mitochondrial density, thus suggesting that low doses of laser irradiation would be more effective in promoting biostimulatory effects.

Concerning on laser and drug absorption, it is well known that the circulatory load of a given area is one of the main factors affecting local absorption from the pharmacological point of view. Recently, Oishi et al. [43] demonstrated that the abdominal acute application of PBM at 660 nm is able to induce a long-lasting hypotensive effect in hypertensive rats and vasodilatation by a NO-dependent mechanism. In the same way, Kazemikhoo et al. [44] demonstrated that it may present a beneficial effect for diabetic patients via decreasing arginase expression and activation of the NOS/NO pathway which increases NO production and vasodilatation, and before that, Plass et al. [45] demonstrated that low-level laser irradiation induced photorelaxation in coronary arteries and overcomes vasospasm of internal thoracic arteries. Taken together, these studies can definitely suggest that laser irradiation is able to produce a local vasodilatation and, in this case, enhance local absorption of topically applied drugs.

From our results, we can conclude that low-level laser therapy (830 nm) was effective in reducing/accelerating the inflammatory process and muscle damage induced by muscle stretching in rats, in addition to providing a significant improvement in functional patterns. Taken together, our results demonstrate the synergistic effect between low-power laser therapy and diclofenac applied by both the topical and IM routes in the experimental model of muscle injury induced by stretching in rats.
Data Availability

All data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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