Effect of ultrasound therapy on the repair of Gastrocnemius muscle injury in rats

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Abstract

The aim of this study was to evaluate the effect of the pulsed ultrasound therapy (PUT) in stimulating myoregeneration and collagen deposition in an experimental model of lacerative gastrocnemius muscle lesion in 30 Wistar rats. Fifteen rats were treated (TG) daily with 1 MHz pulsed ultrasound (50%) at 0.57 W/cm² for 5 min, and 15 were control animals (CG). Muscle samples were analyzed on postoperative days 4, 7 and 14 through H&E, Picrosirius-polarization and immunohistochemistry for desmin. The lesions presented similar inflammatory responses in both treated and control groups. The areal fraction of fibrillar collagen was larger in the TG at 4 days post-operatively (17.53 ± 6.2% vs 6.79 ± 1.3%, p = 0.0491), 7 days (31.07 ± 7.45% vs 12.57 ± 3.6%, p = 0.0021) and 14 days (30.39 ± 7.3% vs 19.13 ± 3.51%, p = 0.0118); the areal fraction of myoblasts and myotubes was larger in the TG at 14 days after surgery (41.66 ± 2.97% vs 34.83 ± 3.08%, p = 0.025). Our data suggest that the PUT increases the differentiation of muscular lineage cells, what would favor tissue regeneration. On the other hand, it is also suggested that there is a larger deposition of collagenous fibers, what could mean worse functional performance. However, the percentage of fibers seems to have stabilized at day 7 in TG and kept increasing in CG. Furthermore, the collagen supramolecular organization achieved by the TG is also significant according to the Sirius red staining results.

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1. Introduction

Despite having different injury mechanisms, the process of muscle repair follows a rather constant pattern. The histopathological pattern of the three phases of the healing process of an injured muscle (destruction, repair, and remodeling) has already been described in detail [1].

Even though the muscle retains its ability to regenerate following injury, muscle healing has been found to be very slow, sometimes, depending on the severity of the muscle injury, with an incomplete muscle recovery. Because fast and complete repair of the injured muscle is the obvious target, one challenging problem in traumatology and in sports medicine is to find clinically feasible treatment modalities that enhance the cell proliferation phase and prevent the occurrence of fibrosis during the reparative process [2].

Therapeutic ultrasound (US) is one of the most frequently used treatment modalities for a variety of skeletal muscle injuries. In spite of over 60 years of a wide range of clinical use, authors affirmed that it is difficult to provide sufficient evidence to establish the clinical efficacy of ultrasound therapy [3]. In vitro studies of fibroblast culture reveal that the benefit of using US could be related to its stimulation on collagen deposition [4] and there are...
experimental evidences that US increases collagen fibers synthesis and organization in tendon repair [5].

Specifically concerning the effectiveness of US for treating muscular injuries, it has been shown that pulsed US enhances both myogenic precursor cell and fibroblast proliferation in an experimental contusion injury to the rat gastrocnemius muscle [6]. Moreover, it has been shown that US treatment improves muscle extensibility [7] and force production after contraction-induced muscle injury [8]. However, other authors using an experimental animal model were unable to demonstrated statistically significant increase of muscle mass, total protein concentration or healing enhancement [10].

Considering the above-mentioned evidences that US stimulates collagen deposition in vivo and in vitro, and also stimulates proliferation of myogenic precursor cells, it was thought to be of interest to proceed to a quantitative evaluation of the balance between myogenic cells and fibrillar collagen in a muscle laceration treated by US, considering that the muscle recovery after injury can turn to be incomplete in cases of excessive collagen deposition [2]. This task was undertaken and, in order to know the tissue area occupied by these tissue components, we applied morphometric techniques in association with the Picrosirius-polarization method according to a classical study in gracilis muscle injury [11] and with an immunohistochemical method for desmin detection [1]. All these techniques were carried out in tissue sections obtained from US treated and control lesions at three post-injury time spans.

2. Materials and methods

2.1. Subjects

The Ethics Committee of the São Paulo Federal University (UNIFESP) reviewed and approved the procedures of this study (# 0300/04). Adult male Wistar rats (approximately 90 days old and weighting 350–400 g) were used. The rats were randomly assigned into six groups (Table 1).

2.2. Surgical procedure

All surgical procedures were carried out under general anesthesia induced by intraperitoneal injection of 100 mg/kg ketamine (Vetbrands, São Paulo, Brazil) and 20 mg/kg xylazine (Agribands, São Paulo, Brazil). All of the hair on the posterior side of the right calf was removed and the skin was incised and retracted. Subcutaneous dissection was performed to permit good exposure of the gastrocnemius muscle. The site of the lesion was standardized for all animals. The gastrocnemius muscle hemitranssection was made at 2.5 cm from the calcaneus flexed at 90°. The laceration was approximately 1.0 cm wide × 0.3 mm deep, located laterally to the vessel-nervous bundle (popliteal artery and tibialis nerve). All measurements were made using a caliper. After controlling the bleeding by compression, the subcutaneous tissue was closed by suture (simple catgut 5.0) and then the skin wound was sutured with stitches of nylon.

After, animals were kept in individual cages with unlimited activities. The room was operated with a 12-h daylight cycle and temperature was maintained at about 25 °C. Food and water were available ad libitum during the study.

2.3. US parameters and treatment

Therapeutic US at 1 MHz with a near field extending for approximately 10 cm from the treatment head, a beam nonuniformity ratio (BNR) below 6.0 and an effective radiating area (ERA) = 3.5 cm² ± 20% was applied; pulses of 5 ms duration were repeated at 100-Hz frequency at a setting of 1:1 (50% duty cycle), with a tissue speed of sound estimated in 1500 m/s. The treatment temporal average intensity was of 0.57 W/cm² for 5 min. This application time was determined because the lesion was smaller than the treatment head [12]. The machine had been calibrated by the manufacturer (Quark, US ProSeven 977 Standard, Brazil). A commercially available US gel was used as a coupling agent and all animals were depilated prior to the application of the ultrasound treatment. (There was thus no fur present between the animal skin and the appliance.) We estimated a tissue thicknesses between the transducer and the focal region of approximated 1.5 cm and a tissue attenuation of 24%/cm. During application, the rat was manually stabilized and care was taken to maintain the proper angle and coupling between the transducer face and tissues. Circular movement of the treatment head was employed to avoid the damages of standing waves.

We compared animals treated with pulsed US daily sessions (beginning 2 days post-trauma) with untreated but operated controls. The number of US sessions for the treated groups is shown in Table 1. The animals were sacrificed at 4, 7 or 14 days post-trauma; five treated and five control animals were sacrificed each time.

2.4. Histology and immunohistochemical study

The gastrocnemius muscles were removed and a fragment containing the injured site was fixed without stretching in 4% paraformaldehyde in PBS. Serial sections (5 μm) were studied using either the Hematoxylin and Eosin
(H&E, for descriptive histopathological studies) or the Picrosirius-polarization method [13]. The latter is a specific histochemical procedure for collagen detection in tissue sections. Briefly, sections were stained for 1 h in a 0.2% solution of Sirius Red, Direct Red 80 (Aldrich Milwaukee, WI 53233) dissolved in aqueous saturated picric acid solution; nuclei were counterstained with hematoxylin. When these tissue sections are observed under polarized light, the enhancement of collagen birefringence promoted by Picrosirius staining is specific for collagen and discloses its distinct patterns of physical aggregation: very thin collagenous fibers (as those present in early granulation tissue) are disclosed as weakly birefringent fibers against a dark background, while thick fibers (characteristic of mature fibrotic lesions) display a strong birefringence [14]. Desmin is a muscle-specific intermediate filament protein expressed since the early formation of skeletal, cardiac and smooth muscle, and endothelial cells. In order to have a precise identification of the muscle lineage cells (from myoblasts to myofibers) in the injured site, tissue sections were submitted to an immunohistochemical reaction for desmin. In brief, after the paraffin wax was removed from the section, microwave pre-treatment (antigen retrieval) was performed. The endogenous peroxidase activity was inhibited and non-specific binding was blocked. Sections were incubated with monoclonal desmin antibody (D1033, Sigma, St Louis, MO, USA) overnight at 4 °C. Reactions were developed using streptavidin-biotin-peroxidase and diaminobenzidine (DAB) (Sigma, St Louis, MO, USA) as used as a cromogen substrate for 10 min at room temperature. Finally the samples were counterstained with Mayer’s Haematoxylin and mounted in permanent mounting medium.

2.5. Morphometry of fibrilar collagen

Slides of regenerating muscle tissue at 4, 7 and 14 days post-trauma of treated and control animals, were analyzed morphometrically to determine the areal fraction occupied by interstitial collagen in the healing tissue [15]. Briefly, the area occupied by collagenous fibers (that appear as deep red fibers in the Picrosirius-stained slides) was selected from a digital image of the total lesion area of each tissue section. The collagen area and the total area of the lesion were calculated using the Image J Software (free software developed at the National Institutes of Health for image analysis); the interstitial collagen areal fraction was then obtained dividing the collagen area by the total injury area.

2.6. Morphometry of regenerating muscle cells

Considering that the aim of this work is to investigate the final effect of US treatment on the imbalance between collagen deposition and myoregeneration, we used the immunohistochemistry stain to analyze, by point counting, the areal fraction of desmin-positive myoblast and myotubes in regenerating muscle tissue of US treated and control animals at 14 days post-trauma. The counting was performed using a coherent test system of 25 points attached to the eyepiece of a light microscope [16]. The analysis was performed at ×200 magnification through all the extension of the healing tissue for each slide. The areal fraction corresponding to myoblasts and myotubes was determined dividing the number of incident points over these cells by the total number of incident points over the healing tissue. Blood vessels were carefully avoided during the measurements because their smooth muscle and endothelial cells are also desmin-positive.

2.7. Statistical analysis

Statistical analysis was performed using Student’s t-test for the average and standard deviation of the morphometric data related to the areal fraction occupied by collagen-containing fibers and myoregenerating cells. F-tests and Shapiro-Wilk tests were carried out previously in order to compare the variances and know if the samples were normally distributed. Statistical significance was defined as $p \leq 0.05$.

3. Results

The three typical zones that characterized the general morphologic pattern of repair evolution of skeletal muscle could be defined in tissue sections of all groups: central zone (CZ), which at early stages contained a hematomata, and which was later filled with granulation tissue; regeneration zone (RZ), where, at early stages, necrosis of myofibers is followed by their nearly complete regeneration, and the surviving zone (SZ) peripherally localized containing stumps of the preserved muscle fibers [1]. These aspects, consistently observed in all lesions studied, are illustrated in Fig. 1A.

3.1. Four days post-trauma

At this stage, it was noted that neither the collagen nor the cellular components of the US treated lesions attain the same structural organization of the control lesions. Although, in both groups, the CZ was filled with vessels and inflammatory cells among some muscle cells showing cytoplasmic degeneration and the RZ was rich of long uninucleated cells (structurally similar to myoblasts), the 4US lesions presented a more developed RZ with some myoblasts fusing into multinucleated myotubes (Fig. 1B). Immunohistochemical analysis revealed numerous desmin-positive myoblasts and myotubes (Fig. 1C and D). These histological differences became apparent when the slides stained with Picrosirius were observed under polarized light. Some occasional spots of thin collagen-containing fibers were observed at the RZ in the 4C group (Fig. 1E), whereas strongly birefringent thick collagen fibers were present in 4US lesions; their orientation were such that they were roughly parallel to each other,
extending up to the CZ (Fig. 1F). It was not possible to identify fibrillar collagen at CZ in 4C or 4US lesions.

3.2. Seven days post-trauma

No major difference could be found between the 7C and 7US lesions stained with Hematoxylin and Eosin. At this post-injury time span, the CZ was smaller than at day 4, and filled with a granulation tissue; the regenerating muscle fibers had begun penetration into the CZ and in some cases, a growth of the myotubes could be seen throughout the region (Fig. 2A). Both 7C and 7US lesions showed regenerative changes, forming a well cellularized connective tissue in the RZ that had a great amount of myotubes (Fig. 2B).

At this post-injury time span, although the amount of collagenous fibers was increased in 7C and 7US lesions, it was still observed that 7C lesions displayed thin, weakly birefringent fibers forming a finely woven meshwork among cellular elements at RZ (Fig. 2C), while the 7US lesions disclosed many thick brilliant (strongly birefringent) collagen fibers at the same localization (Fig. 2D); these thick collagenous septa maintained their roughly parallel orientation. The desmin immunohistochemical staining confirmed the extensive muscle regeneration that occurred in the injured
3.3. Fourteen days post-trauma

At this point, the histological analysis showed that the CZ had contracted and the regenerating borders were closer to each other. When collagen distribution was compared between groups, using the Picrosirius-polarization method, the untreated lesion showed a three dimensional felt work of fibrilar collagen supporting the regenerating muscle cells (Fig. 3A and B) whereas the treated lesion showed a great amount of well oriented thick collagen fibers (Fig. 3C and D). With the aid of desmin immunohistological staining it was clearly observed that the collagen orientation follows the regenerating muscle cells direction, in both groups. There were new myotubes strongly labeled indicating that the process of cellular differentiation was still present at this post-injury time span (Fig. 3E and F).

3.4. Morphometry of fibrilar collagen

The data of the areal fraction occupied by fibrilar collagen (expressed as percentage) in the lesions are shown in Fig. 4. Statistically significant increase was found in the treated groups in all experimental periods compared to respective control groups, i.e., 4 days: 17.53 ± 6.62% vs 6.79 ± 1.32%; p = 0.0491; 7 days: 31.07 ± 7.45% vs 12.89 ± 3.08%; p = 0.0020 and 14 days: 30.38 ± 0.73% vs 19.12 ± 3.51%; p = 0.0119. Statistically significant increase between 4th (6.79 ± 1.32%) and 7th (12.89 ± 3.08%) days, p = 0.0314 and between 7th (12.89 ± 3.08%) and 14th (19.12 ± 3.51%) days, p = 0.0271 in control groups, but significant increase only from 4th (17.53 ± 6.62%) to 7th (31.07 ± 7.45%) days, p = 0.0174 in the ultrasound treated groups.

3.5. Morphometry of desmin-positive myoblasts and myotubes

The data obtained are summarized in Table 2. The statistical analysis showed that the areal fraction of myoregenerating cells (myoblasts and myotubes) was larger at 14 days post-trauma stained with H&E (A), immunostained for desmin (B), and Picrosirius observed under polarized light (C, D). Control (B, C) and ultrasound treated (A, D) lesions. (A) There is still an inflammatory response at the CZ. Note that cells are plentiful in RZ, mainly elongated myotubes which can be characterized by their weakly stained cytoplasm and multiple centrally located nuclei (insert). The arrow indicates a newly formed myotube that has succeeded in extending across the entire lesion gap and fuse to the surviving stumps. (B) Immunohistochemical reactions for desmin reveal a loose connective tissue with numerous myotubes (arrows) among surviving cells (asterisks). (C) Lesions from the control animals at this stage show a loose network of thin fibers of collagenous nature (arrows). (D) Observe that the RZ of ultrasound treated lesions shows thick collagen fibers (red arrows) surrounding groups of cells; almost all these fibers are oriented parallel to each other. Near the CZ, it is possible to identify some newly formed, thin, weakly birefringent collagenous fibers (green arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
days after surgery in US treated lesion compared to control lesions (41.66 ± 2.97% vs 34.83 ± 3.08%, p = 0.025).

4. Discussion

In all post-injury time spans studied, the structural features of the control lesions confirm the description for normal skeletal muscle regeneration when assessing the chronology of repair in the rat skeletal muscle [1]. Considered as a whole, our results suggest a benefit of direct coupling US pulsed in the early post-injury treatment of muscle lacerations.

Muscle lacerations occur when the muscle is transected after direct trauma by a sharp object and there are no guidelines for treatment [10]. Although rare in sports, athletes who experience muscle laceration are incapacitated for long periods of time, and their professional careers are often jeopardized [2]. This study is related to others that aimed to understand the physiopathological basis of the interventions to improve muscle healing after injuries, enhancing muscle regeneration without promoting an excessive scar formation [17,2].

In this study, the Picrosirius stain associated with digital analyses allowed us to demonstrate that the areal fraction occupied by collagen was higher in treated lesions in all post-injury time spans studied. It is interesting to note that, in the control lesions, collagen accumulation kept increas-
In the light of the recent demonstration there is evidence that US accelerate the healing process without an excessive collagenous fibers deposition in bone fractures [18] and in tendon lesions [19]. Considering the fragility of this neoformed tissue, it seems desirable that collagenous fibers could guarantee tissue continuity between the lesion and the surviving tissue, providing nerve and vascular supply, since these fibers do not impair the regenerative process.

The first assessment of the histological effects of US on the skeletal muscle regenerative process after an experimental injury in rats used the incorporation of 5-bromo-2-deoxyuridine to evaluate cell proliferation. These studies showed that US could enhance both the myogenic precursor cell (until 4 days post-trauma) and fibroblast proliferation (at least 10 days post-trauma), and suggested that the prolonged proliferation phase of fibroblasts can add to the amount of permanent scar tissue production. Such effects could outweigh the possible positive effects of US on satellite cell proliferation [6]. It is worth to discuss the criteria used by the authors to distinguish satellite cells from fibroblast. In the light of the recent demonstration there is another distinct population of muscle stem cells located extralaminally which, under stimulus, readily proliferate and give rise to determined myoblasts and differentiate to myotubes [20]. Therefore, part of the proliferating population in the extralaminally compartment – identified by the authors as being fibroblasts – could actually be myogenic stem cells. Thus, they could have overestimated the fibroblast proliferation and, consequently, the scar tissue production.

The Picrosirius-polarization method has already been proved to be useful to establish the degree of the fibrotic lesions by studying the chronological changes in the optical properties of the fibrotic collagen in muscle regeneration in an experimental study of fibrosis in the rat gracilis muscle [11].

The Picrosirius stained sections observed by the aid of polarized light demonstrated that, at days 4 and 7, fibrilar collagen of the regenerative zone of untreated lesions showed up predominantly as thin, weakly birefringent fibers. Strikingly different, the US treated lesion displayed strongly birefringent coarse collagen fibers as early as 4 days post-trauma. Thus, it is possible to suggest that the pulsed US treatment induces the precocious deposition of collagen fibers that display a higher level of supramolecular organization.

Using the immunohistochemical method for identification of Type I and Type III collagen, in a muscle repair study, Lehto et al. [21], showed that the appearance of Type III precedes that of Type I collagen in the injured area in normal muscle repairs. They also showed that, although the labeling for both collagen types increases during the first 5 days in the injured area, the Type III collagen displays a network-like organization pattern at this time, whereas, the bundles of Type I collagen are not detected until 7 days post-trauma. Our results, showing the presence of a weakly birefringent network of thin collagenous fibers (that corresponds to the structural pattern of Type III collagen studied by Picrosirius-polarization method), at 4 days post-trauma control lesions, agree with the foregoing results. Moreover, one of the most important results of the present work was to demonstrate the presence of strongly birefringent bundles of coarse collagen fibers (that corresponds to the structural aspect of Type I collagen fibers studied by Picrosirius-polarization method) in US treated lesions at day 4 post-trauma, which is suggestive that US treatment could stimulate a precocious Type I collagen aggregation.

Since collagen maturation process in the tissue repair area enhances its mechanical strength and resistance to degradation [22] it is possible to suggest that US treated lesions could have a better biomechanical resistance earlier after injuries. The optimal healing of muscle rupture seeks for a balanced regeneration of both the muscle cells and connective tissue components [23]. It is difficult to determine the individual contributions of each biological component to the overall mechanical properties of the muscle repair, once these processes compose a complex scheme. Thus, we suggest that the biomechanical properties derived...
from the different structural arrangement observed in the US treated injury are a field that deserves further study.

Applying a morphometric measure to sections stained (by immunohistochemical methods) for desmin, we could also demonstrate a statistically significant variation in the amount of myoblasts and myotubes: the regeneration zone of US treated lesions displayed a higher amount of myogenic cells when compared to the control lesions at 14th day post-injury. It is important to stress that the morphometric analysis was performed only at this date post-injury because we were interested in a specific muscle tissue evaluation at the furthest time span of the experiment.

To our knowledge, this is the first report demonstrating that, although the pulsed US radiation induced the deposition of collagenous fibers, there was a statistically significant larger amount of myoblasts at 14 days post-trauma in US treated lesions, suggesting that the increase on collagen deposition and aggregation promoted by the US was not enough to impair muscle cells growth and differentiation. Even though achieving these results, we suggest that it is necessary to perform long-term studies with US application, once it is known that the fibrotic scar tissue can be gradually increased in size up to 35 days post-injury [17], which, theoretically, could impair further myogenic cells proliferation [2].

The present results demonstrated that the muscle cells did not attain the same structural organization of the normal muscle in all 14 days post-injury control and US treated lesions. But, even containing thick collagenous fibers in the regenerating zone, it seems that the US treated lesions displayed a better structural arrangement with a more regular alignment of collagen fibers and myotubes. This observation on collagen orientation coincides with the reports in literature showing that the US treated tendon lesion presents the collagen fibers distributed in a more regular orientation [24]. These evidences taken together, and considering the observation that it is not only the amount of collagenous fibers that determines the repair quality, but also their orientation – [25], we can suggest that the US treatment plays a beneficial role in the remodeling of skeletal muscle architecture, resulting possibly in a better biomechanical performance [26,24]. The lack of sufficient evidence to provide a scientific foundation for the clinical use of the US treatment in muscle injuries [10] may be due to a poor clinical study design, inadequate instrument calibration, use of inappropriate coupling medium, poor knowledge of the nature of soft tissue lesions and the presence of more complex underlying pathologies [27]. Thus, the positive results obtained in this controlled experimental work can contribute to the knowledge on the appropriate US parameters for muscle laceration treatment.

The beneficial effect in muscle repair that we found using pulsed US at 0.57 W/cm² agrees with the positive effects in soft tissue regeneration [26]. It is interesting to observe that the frequency of 1 MHz was efficient to improve muscle regeneration, as well as tendon repair [19]. The same can be said of higher frequencies. Current literature demonstrates that ultrasound treatment under US pulsed waves of 1.5 W/cm² and 3 MHz [6], and 1.0 W/cm² and 3.3 MHz [9] are also inefficient to provide myoregeneration.

We followed the indications that the US sessions should be initiated in the early stages of regeneration to obtain its greatest effects: thus, the sessions began in the second day post-injury, and were done daily in order to achieve the US cumulative effect [8,6]. One should consider that, in the present experimental model, the US was administrated using a direct coupling gel method over sutures, which could increase the risk of infection at earlier post-injured time spans in clinical situations.

We chose the pulsed US mode to exclude its thermal effects, based on the successful uses of pulsed US on skin wounds [28], bone fracture repair [18], cartilage healing [29] and in the measurement of in situ range of movements of healing tendon [24]. There are some evidences that the nonthermal effects of US act in several biological processes increasing collagen production [4], membrane permeability [30] and intracellular calcium concentration [31]. The pulsed mode at 50% also seems to be more favorable towards myogenesis than a duty cycle of 20% used by other authors [6,9], since they did not obtain a myotube formation as favorable as in the present study.

The events of tissue regeneration occur not only as a response to the biochemical inflammatory stimulus but also to mechanical signals, such as the US stimulus. As presented earlier, the network of signaling pathways (involving the activation of β1 integrins and RhoA/ROCK-and Src-ERK signaling cascade) that is triggered by pulsed US may induce skin fibroblast proliferation in vitro [32]. In this model the integrins seem to act as mechanotransducers to transmit pulsed US energy into intracellular biochemical signals inducing cell activation. It is possible that the satellite cells proliferation could also be stimulated by the mechanical effect of the US.

It is important to emphasize that our data are not sufficient to indicate pulsed US application to the muscle lesions without restriction, since the striated muscle optimal repair requires not only a balanced interaction between the regenerating muscle cells and extracellular matrix, but also the neovascularization and the adhesion of the myofibers to the extracellular matrix [33]. In order to definitely determine the effects of US on muscle injuries, all these parameters must be analyzed in relation to the different kinds of muscle injury and repair process stages. Moreover, to correctly answer these questions, biomechanical studies should be performed to establish the properties of this new tissue, since it is well known that small structural variations can be responsible for considerable biomechanical differences. We hope that our observations, when coupled to future biomechanical data concerning the effects of US treatment on skeletal muscle, may help to explain some of the complications of the muscle healing process.
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References


