Can Electroacupuncture Enhance Biomechanical and Histological Analysis after Experimental Muscular Contusion?

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Abstract

Considering that inflammatory course may occur in the muscle healing process after injury and that there is a lack of scientific evidence for the use of acupuncture as a treatment to enhance skeletal muscle regeneration, the aim of this study was to evaluate if microscopic events can interfere with macroscopic effects of electroacupuncture in an animal muscle contusion model. It was hypothesized that this treatment is effective in this injury. Seventy-eight Wistar rats were divided in 7 groups: control; 3-days and 5-days injury, treatment and placebo groups. The treatment improvement was compared between groups by biomechanical and histological analyses. In maximum load, no differences were finding between 3-days injury, placebo and treatment groups. No difference was observed between 5-days groups. In stiffness, no differences were observed between all groups. The histological analysis showed some slides of injured group with areas of evident bruising. In sham and treatment group were also areas of bruises but in process of advanced repairing. The results suggest a histological and functional improvement with the use of electroacupuncture, but there was no biomechanical improvement. It was not possible to see differences between the treated group and placebo. Our results support that there is no biomechanical evidence that electroacupuncture enhances skeletal muscle regeneration after contusion injury. In contrast, histological analysis supports a contrary idea of this fact. Besides the contradictories results of our work, more studies must be done to provide support to electroacupuncture as a treatment, especially in muscular injuries.

Keywords: electroacupuncture, skeletal muscles, injuries, contusions, biomechanics, histology, rats, tensile strength

1 Introduction

Muscle injuries account for up to 30% of the injuries sustained in sports events [6]. More than 90% of these muscle injuries are caused either by contusion or by excessive strain of the muscle [15,16]. In professional sport, any of these injuries can lead to significant pain and disability causing loss of training and competition time.

Although the muscle is able to regenerate, recurrent muscle injuries could affect muscle performance. Skeletal muscle regeneration has been well documented [4] and several studies have shown that skeletal muscles regenerate after contusion [9,12,27,30].

Even though contusion injuries are a very common form of both athletic and nonathletic injury, hospitalization is rarely required. However, these injuries affect muscle function. Structural and functional morbidity often occurs in the form of atrophy, contracture, pain, and increased likelihood of reinjury [26,38].

The healing process of damaged muscle tissue is characterised by three phases: the initial destruction phase with concomitant haematoma formation, myofiber
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necrosis, and inflammation; the repair phase, consisting of phagocytosis of the necrotised tissue and regeneration of the myofibers by activated and proliferating satellite cells; the remodelling phase, where the regenerated myofibers mature and the function of the repaired muscle is restored [22].

Besides this process is regulated in part by growth factors and cytokines, which are locally produced and released from injured myofibers and inflammatory mononuclear cells at the site of muscle injury [23]. There is direct evidence that tumor necrosis factor–α (TNF-α) has a physiological role in the regeneration of the injured skeletal muscle, as the inhibition of its activity during the healing results in a slight deficit in the strength of the recovering skeletal muscle [9,39]. Furthermore, a large number of growth factors and cytokines, such as the members of fibroblast growth factor, insulin-like growth factor (IGF), and transforming growth factor–β (TGF-β) families; hepatocyte growth factor (HGF); and interleukin-1β (IL-1β) and IL-6, are known to be expressed in the injured skeletal muscle, and it is likely that several other growth factors such as the platelet-derived growth factor are also expressed in the injured muscle [1,5,32] at least their expression can be induced in the skeletal muscle by such physiological stimuli (that actually causes micro-traumas) as external stretching or mechanical loading [25,32].

Treatments to augment the normal repair and regenerative processes are important to a wide variety of patients, ranging from elite athletes to the elderly [2,3,13,28,29,31,33,34], who want to return to their previous level of function as quickly and as fully as possible.

Acupuncture treatment is initiated as a complementary therapy and replacement in some cases a conventional pharmacological intervention. A number of observations on the anti-inflammatory actions of acupuncture have been published, representing open studies and randomised trials [11,41]. The direct and indirect effects of individual neuropeptides, cytokines (TNF-α, IL-1β, IL-6 and others) and vasoactive mediators could be considered to play an intermediate role during and after acupuncture treatment.

Considering that inflammatory course may occur in the muscle healing process after injury and that there is a lack of scientific evidence for the use of acupuncture as a treatment to enhance skeletal muscle regeneration, the aim of this study was to evaluate if microscopic events can interfered with macroscopic effects of electroacupuncture in an animal muscle contusion model. It was hypothesized that this treatment is effective in this injury.

2 Materials and Methods

Seventy-eight adult female Wistar rats (University of São Paulo, Ribeirão Preto, SP, Brazil), mean weight of 255.75 ± 21.77 g, were used in this study. All animals were housed separately in number of five and had food and water ad libitum. All experimental protocols were approved by the Institutional Animal Care and Use Committee at our institution (CETEA). In all animals, a bilateral muscle contusion injury was created in the gastrocnemius muscle. All procedures
were performed under anaesthesia with ketamine (100 mg/kg) and xylazine (40 mg/kg) and at the end of the experiments the rats were humanely sacrificed by chloral-hydrate overdose.

Under anesthesia, both hind limbs were placed in a specially designed device, adapted by Crisco [9] (Figure 1). For our study, this device was slightly modified to accommodate the small size of the animals. The limbs were secured in a holding device to position the midportion of the gastrocnemius muscle exactly under the impactor tip. The ankle was placed in 90° of dorsiflexion to create tension in the gastrocnemius muscle.

An impactor with a round 5-mm-diameter tip was allowed to rest on themidportion of the muscle belly. A 200 g weight was dropped from a 30 cm height through an acrylic guide tube. The weight dropped onto the impactor, driving it into the muscle belly without injuring the overlying skin. In other studies, this action resulted in a contusion and damage to the muscle fibers with a subsequent inflammatory response but no macroscopic rupture [10].

The animals were divided into 7 groups: control (C) (3 plus 5-days contralateral muscles to injury groups), 3 and 5 days lesion (L), treatment (T) and sham (simulated) (S) groups. The animals in group L (n=16) were injured. In group C, the opposite limbs to the lesion were used. The impacted animals in group T (n=16) were injured and received electroacupuncture for 5 days beginning immediately after the injury. Group S (n=16) were injured and received a sham electroacupuncture. The animals were evaluated day 3 and 5 after the injury but 5-days histological analysis was done.

Rats were bilaterally stimulated at ST36 and SP6 points. ST36 lies just 0.5 cm below fibular head of hinder leg in rat, SP6 just 0.5 cm above medial malleolus. The non-acupoint or sham group were done at 0.5 cm beside ST36 and SP6 [14]. A point locator was used. These rats were immobilized, and then were stimulated by the intermittent pulse with 2Hz frequency for 15 minutes once a day and 5 times in all [35]. The electroacupuncture initiated immediately after the injury. A manual contention of the rats with no anesthesia was used. No signs of stress of the rats were observed.

The animals were subsequently sacrificed, and gastrocnemius muscle was harvested by carefully opening the skin above the muscle on the middle side. The muscle was carefully dissected in a standard fashion, removed, and immediately weighed. After weighting, 5-days muscles (n=6) was fixed in 10% formaldehyde (seven days) for histological evaluation. The muscle was sectioned transversally into 2 halves directly through the site of injury (middle zone). Histological slices (6 µm) of the cut edge were stained with hematoxylin and eosin. Others gastrocnemius muscles (n=10) was situated in your complex (femur-gastrocnemius-calcaneus) for accomplishment of the mechanical tests to evaluate the tensile properties of the skeletal muscle.

For the mechanical tests, a Universal Testing Machine (EMIC®) (Figure 2) with a load cell capacity of 500 N was used. According to an established methodology of our Laboratory, a preload of 5 N, was applied for a time of accommodation of 30 seconds (Figure 3). Besides, a load rate of 10 mm / minute was established, until the rupture of the muscle. Maximum load and relative
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stiffness were evaluated.

All data are given as (mean ± SD). PrismPad Graph 5 software was used to perform all statistical tests. Statistical comparisons between groups were carried out using ANOVA test, Tukey post-test and p < 0.05 was set to indicate statistical significance.

3 Results

3.1 Body and muscle weight

Table 1 represents all values of the body and muscle weight.

No difference was observed between final body weights.

The results of this study showed 3-day-injured muscles were heavier than control ones, treated and simulated groups. 5-days muscles did not show any differences between groups.

3.2 Mechanical properties

Table 1 also represents values of the mechanical properties.

In maximum load (Graph 1), no differences were finding between 3-days injury, placebo and treatment groups. No difference was observed between 5-days groups.

In stiffness, no differences were observed between all groups (Graph 2).

3.3 Histological findings

Five days after contusion, hematomas were present with sites of basofilia, probably representing fibers in regeneration. Injury and placebo groups showed this behavior (figures 4 and 5), but we found treatment group with as advanced reparation (figure 6).

4 Discussion

Although the medical community has made efforts to decrease such morbidity, the best treatment of a muscle contusion injury has yet to be defined [13,34].

There are an enormous variety of possible treatments for muscular injuries [9]. Immobilization and remobilizations [20,21], RICE - “rest, ice, compression and elevation” [3,36], nonsteroidal anti-inflammatory [31,33] or corticosteroids drugs [2], therapeutic ultrasound [28,34], and others, are recent treatments used to promote a faster healing process.

It is theorized that electroacupuncture may influence cellular function because it may lead to activation of signal-transduction pathways involved in healing. Electroacupuncture stimulus may cause cytokines and growth factors inhibition or
Excitation of the multimolecular complex of muscular regeneration. Furthermore, there are no data available on clinical effects, if any, of electroacupuncture on regeneration after contusion muscular.

Electroacupuncture is not a commonly prescribed treatment for contusions. However, this technique was largely used as treatment for painful, inflammatory and chronic diseases [7,11,24,35,37,40]. Thus, our purpose in performing this research was to use a reproducible drop-mass technique to create a standardized skeletal muscle contusion injury, in order to examine the effects of the electroacupuncture on muscular regeneration.

We documented an increase in muscle weight after 3-days injury, which we attributed to an accumulation of edema and hemorrhage. Järvinen [21] recorded an increase in the weight of the injured muscle 2 days after injury, with a slight decrease in weight therefore. Fisher et al., [12] found no increase in the weight of the injured muscle and suggested protein loss equaled the increased weight to edema e hemorrhage.

The effects of contusion injury and healing has been quantified by the passive tensile failure properties of the rats gastrocnemius muscle [21]. They showed that passive failure load significantly decreased after injury and continually improved with healing but at 42 days after injury remained 95% of normal [9,21].

Our results suggest that the intervention did not alter the mechanical properties after contusion injury. We were unable to demonstrate any significant trends in the maximum load or relative stiffness. The differences with our results may to be due to the more serious injury model of Järvinen and Sorvari [20]. However in agreement with their studies, we also observed that failure initiated at the site of the injury acutely and during the early stages of healing [9,20].

Given that results passive failures is an indication of tissue strength [15], this results suggest that acute contusion injuries may be more susceptible to subsequent strain injuries at the site of injury [9]. However, our results showed an interesting behavior observing relative stiffness. The muscular tissue probably provides structural protection for subsequent strain injuries because it maintains stiffness of this system equally independently of the injury, i.e. the muscle responds to a load with a similar deformation in injury or noninjury. Nevertheless there are differences in peak loads.

Our study also find in histological analysis, injured and placebo group with areas of evident bruising. In electroacupuncture group there were also hematoma areas but in process of advanced repair, the tissue was apparently more highly organized and appeared much closer to normal than injuries muscles.

5 Conclusions

Our results support that there is no biomechanical evidence that electroacupuncture enhances skeletal muscle regeneration after contusion injury. In contrast, histological analysis supports a contrary idea of this fact. Besides the contradictories results of our work, more studies must be done to provide support to electroacupuncture as a treatment, especially in muscular injuries.
Acknowledgements.
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References


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FIGURES, GRAPHS AND TABLES

Figure 1: Schematic of impact apparatus.

Figure 2: Universal Testing Machine EMIC®, DL 10000 model
Figure 3: Gastrocnemius muscle fixation in the Universal Testing Machine.

Table 1: Body and muscle weight, maximum loads and relative stiffness averages were presenting for all experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final Body Weight (g)</th>
<th>Gastrocnemius Muscle Weight (g)</th>
<th>Maximum Load (N)</th>
<th>Relative Stiffness (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>252,5 ± 21,7</td>
<td>± 1,76 ± 0,09</td>
<td>37,21 ± 4,2</td>
<td>4,30 ± 0,79</td>
</tr>
<tr>
<td>3-days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion (GL3)</td>
<td>251,0 ± 16,1</td>
<td>± 2,07 ± 0,14</td>
<td>32,12 ± 2,68</td>
<td>3,72 ± 0,48</td>
</tr>
<tr>
<td>Treatment (GT3)</td>
<td>271,0 ± 11,9</td>
<td>± 1,77 ± 0,10</td>
<td>29,93 ± 5,23</td>
<td>3,83 ± 0,90</td>
</tr>
<tr>
<td>Simulated (GS3)</td>
<td>255,0 ± 13,1</td>
<td>± 1,61 ± 0,09</td>
<td>31,78 ± 3,71</td>
<td>4,25 ± 0,69</td>
</tr>
<tr>
<td>5-days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion (GL5)</td>
<td>259,0 ± 12,6</td>
<td>± 1,69 ± 0,10</td>
<td>36,02 ± 6,26</td>
<td>4,11 ± 1,17</td>
</tr>
<tr>
<td>Treatment (GT5)</td>
<td>260,0 ± 21,1</td>
<td>± 1,64 ± 0,09</td>
<td>36,37 ± 4,98</td>
<td>4,74 ± 1,14</td>
</tr>
<tr>
<td>Simulated (GS5)</td>
<td>253,5 ± 20,3</td>
<td>± 1,61 ± 0,14</td>
<td>36,34 ± 5,33</td>
<td>4,50 ± 0,71</td>
</tr>
</tbody>
</table>
Graph 1: Average maximum loads of experimental groups. Control group (GC), 3-days lesion (GL3), treatment (GT3), sham (GS3) groups and 5-days lesion (GL5), treatment (GT5), sham (GS5) groups. No differences were found between 3-days lesion group and 3-days treatment and sham groups. 5-days showed similar behavior.

Graph 2: Average relative stiffness of experimental groups. Control group (GC), 3-days lesion (GL3), treatment (GT3), sham (GS3) groups and 5-days lesion (GL5), treatment (GT5), sham (GS5) groups. No differences were found between all groups.
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Figure 4: Transversal section showing gastrocnemius muscular fibers of 5-days injury group. We observed the hematoma in injury area (arrow). 10X image caption and 1.0 optovar. H&E staining.

Figure 5: Transversal section showing gastrocnemius muscular fibers of 5-days electroacupuncture group. We observed the hematoma in injury area (arrow) in an advanced process of repairing. 10X image caption and 1.0 optovar. H&E staining.
Figure 6: Transversal section showing gastrocnemius muscular fibers of 5-days placebo group. We observed the hematoma in injury area (arrow). 10X image caption and 1.0 optovar. H&E staining.

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