Physiotherapy 97 (2011) 163–169

Haematological effect of pulsed ultrasound in acute muscular inflammation in rats

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Abstract

Objective To evaluate the effect of low-intensity pulsed ultrasound (LIPUS) on the haematological dynamics of an acute inflammatory process after an iatrogenic muscular lesion.
Design Controlled laboratory study.
Setting Research laboratory.
Participants Eighteen male Wistar rats (weight 350 to 450 g) were submitted to surgical incision in the biceps femoris muscle (approximately 50%), and subsequently subdivided into control ($n = 9$) and LIPUS ($n = 9$) groups.
Intervention Ultrasound (1.0 MHz) was applied at the lesion site in the pulsed mode (2 ms on, 8 ms off) at 0.4 W/cm^2 for 3 minutes at 1, 8 and 24 hours post-surgery.
Main outcome measures In these periods, blood was collected through venepuncture of the retro-orbital plexus and evaluated for different types of leukocytes and erythrocytes.
Results LIPUS reduced the total leukocyte count at 1, 8 and 24 hours post-surgery (1 hour, control $9017.2 \pm 481 \times 10^3$/mm^3 vs LIPUS $6189.8 \pm 450 \times 10^3$/mm^3; 8 hours, control $8078.2 \pm 501 \times 10^3$/mm^3 vs LIPUS $5371.3 \pm 378 \times 10^3$/mm^3; 24 hours, control $8192.3 \pm 646 \times 10^3$/mm^3 vs LIPUS $6059.1 \pm 503 \times 10^3$/mm^3; $P < 0.001$). The monocyte count was reduced at 8 and 24 hours post-surgery (8 hours, control $815.5 \pm 126 \times 10^3$/mm^3 vs LIPUS $375.4 \pm 70 \times 10^3$/mm^3; 24 hours, control $875.3 \pm 124 \times 10^3$/mm^3 vs LIPUS $564.7 \pm 56 \times 10^3$/mm^3; $P < 0.001$). The number of segmented neutrophils was only reduced at 1 hour post-surgery (control $5033.1 \pm 397 \times 10^3$/mm^3 vs LIPUS $3594.8 \pm 191 \times 10^3$/mm^3; $P = 0.006$), and the lymphocyte count was only reduced at 8 hours post-surgery (control $4759.7 \pm 459 \times 10^3$/mm^3 vs LIPUS $2584.1 \pm 356 \times 10^3$/mm^3; $P = 0.003$). Changes were not observed in the concentrations of young neutrophils, polibocytes and erythrocytes.
Conclusion LIPUS reduced aspects of the inflammatory process following an acute incisional muscular lesion.
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Keywords: Inflammation; Myoregeneration; Rehabilitation; Ultrasonic therapy; Wounds and injuries

Introduction

Skeletal muscle injuries are common in clinical sports medicine. Although there is little scientific evidence, therapeutic ultrasound is widely recommended and is used in the treatment of muscle injuries [1]. Conversely, a number of animal studies have suggested the beneficial effects of

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0031-9406/$ – see front matter. Published by Elsevier Ltd on behalf of Chartered Society of Physiotherapy
doi:10.1016/j.physio.2010.06.004

low-intensity pulsed ultrasound (LIPUS) in wound repair of tissues other than muscle [2,3], skin [4], bone [5], tendon [6] and ligament [7]. LIPUS is widely used to accelerate tissue regeneration following injury, but the biological mechanisms for this effect are poorly understood [4].

Injury results in the rapid necrosis of myofibres and the activation of inflammation, which contributes to the removal of necrotic material and the secretion of several cytokines and growth factors stimulating satellite cell activation [8]. Immediately after an injury to skeletal muscle, the gap formed between the ruptured muscle fibres is filled with a haematoma, where macrophages and fibroblasts are activated, producing additional chemotactic signals (e.g. growth factors, cytokines and chemokines) for the circulating inflammatory cells [1]. The injured myofibres undergo necrosis by autodigestion mediated by intrinsic proteases [9].

Inflammation is clearly a critical component of muscle physiology, and is an important phase in the regenerative process where the goal of treatment is to limit the size of the haematoma and avoid an excessive inflammatory reaction [8]. Functional impairment is associated with perturbed spatial distribution of inflammatory cells, altered identity of the inflammatory infiltrate (cell type and magnitude of influx) and the temporary sequence of these events [8]. On the first day after injury, inflammatory cells including phagocytes invade the haematoma and begin to remove the blood clot [1]. The necrotic area is invaded by small blood vessels, and mononuclear cells, activated macrophages and T-lymphocytes infiltrate the local tissue. These activated lymphocytes simultaneously secrete several cytokines and growth factors which perform a wide range of functions in the inflammation process [10]. For instance, there is an increase in the blood concentration of polymorphonuclear leukocytes (neutrophils, eosinophils and basophils), particularly neutrophils [1,11,12], and this is followed by monocytosis [13]. The level of erythrocytes remains stable but, depending on the lesion type and extent, can decrease because of imprisonment of erythrocytes in the white thrombus (rich in platelets) and due to haemorrhage [14].

The authors' group recently demonstrated that continuous ultrasound in the acute phase of iatrogenic muscle injury promotes erythrocyte reduction and an increase in the concentrations of segmented neutrophils and eosinophils. These modifications suggest increased haemorrhage and an amplified inflammatory response following acute muscle injury [15]. On the other hand, the LIPUS referred effects are decreased wound size, increased collagen deposition, increased ability to withstand tensile loading [16], stimulation of the production of angiogenic factors [17], and increased concentrations of infiltrated neutrophils and monocytes inside and between the basal laminae [2]. Although use of non-thermal therapeutic ultrasound remains widespread in the clinical treatment of muscular injuries, there is a growing body of literature that questions its effectiveness [18,19]. However, LIPUS (frequency 1 MHz, intensity 0.5 W/cm^2, duty cycle 20%) increased the mechanical properties of the injured muscles [20] and, when applied in the first hours after muscular lesion, altered the transduction of cell signalling through modifications in oxidative stress [21], suggesting that parameters of similar applications affect the haematological dynamics in favour of myoregeneration. The aim of this study was to evaluate the effect of LIPUS on the haematological dynamics of different types of leukocytes and erythrocytes after acute muscle injuries.

Materials and methods

Subjects

Animal manipulation was performed in accordance with the animal testing guide, and this study was reviewed and approved by the Research Ethics Committee of the University of Cruz Alta (UNICRUZ/Rio Grande do Sul, Brazil). All animals were maintained on a 12-hour dark/light cycle at 20 to 24 °C and relative humidity of approximately 50%. Food and water were given *ad libitum* throughout the experimental protocol. The animals' maturation time was 30 weeks. Eighteen mature male Wistar rats (weight 300 to 400 g) were used in this study. The rats were randomised before surgery to the control group (submitted to the injury protocol and the therapeutic procedure with the ultrasound equipment turned off; $n = 9$) and the LIPUS group (submitted to the injury protocol and ultrasonic therapy; $n = 9$). The groups were submitted to surgical incision on the lateral aspect of the right hind limb according to the injury protocol.

Injury protocol

The animals were anaesthetised with a combination of xylazine (7 mg/kg) and ketamine (70 mg/kg) administered intraperitoneally. A longitudinal surgical incision was made on the skin of the right hind limb to facilitate subcutaneous tissue rupture and to provide easy access to the middle portion of the biceps femoris muscle. Approximately 50% of muscle fibres were transversally incised. The skin lesion was subsequently closed by surgical suture. This muscle was chosen due to its easy access in rats, and the adequate distance from bone structures which could indirectly interfere with the therapeutic ultrasound stimulus [15].

Ultrasound treatment

After surgery, the rats in the LIPUS group were treated with ultrasound, applied directly to the injured area. A commercially available ultrasound gel was used as a coupling agent, and all animals were depilated prior to application of the ultrasound treatment. AVATAR V (Model 9075, Biosistemas Equipamentos Eletrônicos Ltda, Amparo, São Paulo, Brazil) was used, calibrated by the manufacturer before the study, with the radiant force method. Ultrasound was applied