Cell Biology International 31 (2007) 482–488

Cell Biology International

www.elsevier.com/locate/cellbi

Effect of therapeutic pulsed ultrasound on parameters of oxidative stress in skeletal muscle after injury

Luciana S. Freitas [a], Tiago P. Freitas [a,b], Paulo C. Silveira [b], Luís G. Rocha [b], Ricardo A. Pinho [b], Emilio L. Streck [a,*]

[a] *Laboratório de Fisiopatologia Experimental, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil*
[b] *Laboratório de Fisiologia e Bioquímica do Exercício, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil*

Received 2 October 2006; revised 3 November 2006; accepted 16 November 2006

Abstract

Contusion injuries are a very common form of both athletic and non-athletic injury, that effect muscle function. Treatments to augment the normal repair and regeneration processes are important for a wide variety of patients. Therapeutic ultrasound has been claimed to promote tissue repair, especially by enhancing cell proliferation and protein synthesis. The present study aimed to investigate the effect of therapeutic pulsed ultrasound (TPU) on parameters of oxidative stress, namely thiobarbituric acid-reactive substances (TBARS), protein carbonyl content and the activities of antioxidant enzymes, catalase and superoxide dismutase (SOD), in skeletal muscle after injury. Wistar rats were submitted to an animal model of muscle (gastrocnemius) laceration. TPU was used once a day. One, three or five days after muscle laceration, the animals were killed by decapitation and oxidative stress parameters were evaluated. Serum CK levels were increased in muscle-injured animals, indicating that the laceration animal model was successful. TBARS were not altered after muscle injury, when compared to the sham group. Protein carbonyl content was increased after muscle laceration. Catalase and SOD activities were increased 1 day after muscle injury and not altered at days 3 and 5. TPU decreased TBARS levels after muscle laceration when compared to injured muscle animals without treatment. Protein carbonyl content evaluation presented similar results. It is tempting to speculate that TPU seems to protect the tissue from oxidative injury. TPU diminished catalase and SOD activities, especially on the first day following muscle laceration.

Keywords: Therapeutic pulsed ultrasound; Muscle injury; Oxidative stress

1. Introduction

Contusion injuries are a very common form of both athletic and non-athletic injury, that effect muscle function. Treatments to augment the normal repair and regeneration processes are important for a wide variety of patients, ranging from athletes to the elderly, who want to return to their previous level of function as quickly and as fully as possible (Karnes and Burton, 2002). Muscle injuries occur by a variety of mechanisms, such as direct forces, including muscle lacerations and contusions, indirect forces related to strains (Crisco et al., 1994; Hughes et al., 1995; Kasemkijwattana et al., 1998a,b; Menetrey et al., 1999, 2000) or as delayed-onset muscle injuries caused by intensive exercise (Garrett, 1990; Lehto and Järvinen, 1991; Fridén and Lieber, 1992).

Injured muscle usually undergoes a healing process of degeneration and regeneration comprising three phases (Li et al., 2001; Huard et al., 2002). In the first phase, inflammation, the necrosis of damaged myofiber segments and phagocytosis of the damaged tissue by macrophages are the main features. This phase is characterized by formation of a hematoma, necrosis of muscle tissue, degeneration, and inflammatory cell response. The main goal of the treatment in this phase is to limit the size of the hematoma and excessive

* Corresponding author. Tel.: +55 48 3431 2539.
E-mail address: emiliostreck@terra.com.br (E.L. Streck).

1065-6995/$ - see front matter © 2006 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.cellbi.2006.11.015

inflammatory reaction (Worrell, 1994). After initial muscle injury, oxidative stress could be increased by the presence of neutrophils and macrophages in muscles. The production of reactive oxygen species by such infiltrates can damage lipid membranes and produce cell necrosis (Halliwell and Chirico, 1993). After some days the second phase takes place. The main feature of the phase of repair (or regeneration) is proliferation of reserve satellite cells and endomysial fibroblasts, followed by active protein synthesis.

Intrinsic factors, such as the components of the disintegrated extracellular matrix and plasmalemma and the availability of growth factors, control these processes (Grounds, 1991; Bischoff and Heintz, 1994). The regeneration of the striated muscle, production of connective tissue scar and capillary growth also occur (Carlson and Faulkner, 1983; Alameddine et al., 1989; Grounds, 1991). The third phase of muscle healing, known as maturation or remodeling, is characterized by a gradual recovery of the functional properties of the muscle, including the recovery of the tensile strength of its connective tissue component (Lehto and Järvinen, 1991). In this phase, the regenerated muscle matures and contracts with reorganization of the scar tissue (Lehto et al., 1986; Menetrey et al., 1999). Even though the new muscle fibers have the ability to regenerate after injury, the healing process tends to be very slow and often leads to incomplete functional recovery. Fast and complete repair of the injured muscle (diminution of the time of recovery of the injuries) is the obvious target, especially in athletes (Menetrey et al., 1999; Fukushima et al., 2001; Li et al., 2001; Huard et al., 2002).

In this context, therapeutic ultrasound is a commonly used mode of physical therapy with the objective of enhancing repair of tissue injuries and to reduce associated pain (Van der Windt et al., 1999; Warden, 2003; Järvinen et al., 2005). However, there are few data showing that ultrasound assists in skeletal muscle regeneration. Therapeutic ultrasound has been claimed to promote tissue repair, especially by enhancing cell proliferation and protein synthesis during the healing of skin wounds (Webster et al., 1980), tendon injuries (Frieder et al., 1988; Enwemeka et al., 1990) and fractures (Duarte, 1983; Heckman et al., 1994). Because of the lack of scientific evidence, the use and prescription of therapeutic ultrasound as a treatment to enhance skeletal muscle regeneration is often based on the personal opinions and experience of clinicians (Van der Windt et al., 1999; Warden, 2003; Järvinen et al., 2005). There is still no consensus statement on the appropriate parameters for treatment of muscle injuries or whether use of therapeutic ultrasound is even justified as a treatment when the goal is to influence skeletal muscle repair and regeneration.

Oxidative stress could be especially increased in the inflammatory phase of the muscle healing process. The presence of neutrophils and macrophages in muscles may lead to overproduction of reactive oxygen species. These species may damage lipid membranes and produce cell necrosis (Halliwell and Chirico, 1993). In this context, lipid peroxidation appears to be an important mechanism underlying exercise-induced muscle damage. Although it has been demonstrated that antioxidant enzymes increase following oxidative stress and exercise training (Duthie et al., 1990; Gohil et al., 1988), the increase in antioxidant defenses might not be physiologically proportionate to the needs created by the increase in prooxidant events.

Considering that oxidative stress may occur in the muscle healing process after injury and that there is a lack of scientific evidence for the use of therapeutic ultrasound as a treatment to enhance skeletal muscle regeneration, the present study aimed to investigate the effect of therapeutic pulsed ultrasound (TPU) on parameters of oxidative stress, namely thiobarbituric acid-reactive substances (TBARS), protein carbonyl content and activity of the antioxidant enzymes, catalase and superoxide dismutase, in skeletal muscle after injury.

2. Methods

2.1. Animals

Male Wistar rats obtained from the Central Animal House of Universidade do Extremo Sul Catarinense were caged in groups of five and provided with commercial rat chow and water *ad libitum* and maintained on a 12 h light/12 h dark cycle. The animals were divided into five groups: (1) sham group (uninjured muscle animals); (2) control of muscle injury without treatment; (3) muscle injury and TPU (0.5 W/cm^2); (4) muscle injury and TPU (1.0 W/cm^2); (5) muscle injury and TPU (2.0 W/cm^2). All studies were performed in accordance with National Institutes of Health guidelines and with the approval of the Ethics Committee from Universidade do Extremo Sul Catarinense.

2.2. Muscle laceration

The muscle laceration model was described by Menetrey et al. (1999) and Chan et al. (2003). The rats were anesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (15 mg/kg). After that, a posterior longitudinal skin incision was made at the calf area, and a subcutaneous dissection was performed to permit open exposure of the gastrocnemius muscle. Gastrocnemius muscles were cut at 60% of the length from their distal insertion, through 75% of their width and 50% of their thickness. After controlling the bleeding with cauterization or simple compression, muscles were sutured with a modified Kessler stitch and simple sutures using a polydioxanone 7.0 wire.

2.3. Therapeutic pulsed ultrasound

After 12 h of muscle injury, TPU was used for groups 3, 4 and 5. After that, TPU was used once a day. One, three or five days after muscle laceration, the animals were killed by decapitation. The injured region of the muscle (gastrocnemius) was removed and the blood was collected. By using this experimental design, we could evaluate the progress of muscle healing without treatment (group 2) compared to uninjured muscle animals (group 1) and the effect of TPU in different doses (groups 3, 4 and 5) compared to injured muscle animals without treatment (group 2) on oxidative stress parameters in skeletal muscle of rats.

2.4. Sample preparation

Gastrocnemius was homogenized in the buffer used for each technique. The homogenates were centrifuged at 1000 × *g* for 10 min at 4 °C and the supernatants kept at −70 °C until used for the experiments. The maximal period between homogenate preparation and biochemical analysis was always less than 5 days. Protein content was determined by the method described by Lowry et al. (1951) using bovine serum albumin as standard.