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Effects of cryotherapy combined with therapeutic ultrasound on oxidative stress and tissue damage after musculoskeletal contusion in rats

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Abstract

Objective To investigate the combined effects of cryotherapy and pulsed ultrasound therapy (PUT) on oxidative stress parameters, tissue damage markers and systemic inflammation after musculoskeletal injury.
Design Experimental animal study.
Setting Research laboratory.
Participants Seventy male Wistar rats were divided into five groups: control, lesion, cryotherapy, PUT, and cryotherapy + PUT.
Interventions The gastrocnemius muscle was injured by mechanical crushing. Cryotherapy was applied immediately after injury (immersion in water at 10 °C for 20 minutes). PUT was commenced 24 hours after injury (1 MHz, 0.4 W/cm^{2SPTA}, 20% duty cycle, 5 minutes). All animals were treated every 8 hours for 3 days.
Main outcome measures Oxidative stress in muscle was evaluated by concentration of reactive oxygen species (ROS), lipid peroxidation (LPO), anti-oxidant capacity against peroxyl radicals (ACAP) and catalase. Plasma levels of creatine kinase (CK), lactate dehydrogenase (LDH) and C-reactive protein (CRP) were assessed.
Results When applied individually, cryotherapy and PUT reduced CK, LDH, CRP and LPO caused by muscle damage. Cryotherapy + PUT in combination maintained the previous results, caused a reduction in ROS [$P = 0.005$, mean difference -0.9×10^{-8} relative area, 95% confidence interval (CI) −0.2 to −1.9], and increased ACAP {$P = 0.007$, mean difference 0.34 1/[relative area with/without 2,2-azobis(2-methylpropionamidine)dihydrochloride], 95% CI 0.07 to 0.61} and catalase ($P = 0.002$, mean difference 0.41 units/mg protein, 95% CI 0.09 to 0.73) compared with the lesion group.
Conclusions Cryotherapy + PUT in combination reduced oxidative stress in muscle, contributing to a reduction in adjacent damage and tissue repair.

Keywords: Rehabilitation; Skeletal muscle; Inflammation; Physical therapy modalities; Oxidative stress; Ultrasonic therapy

Introduction

Muscle injuries are one of the main traumas that occur when participating in physical and sporting activities [1,2]. Inflammation is the most important phase (1 to 3 days) in the muscle repair process because it determines the extent and duration of recovery. During this period, there is an increase in the concentration of reactive oxygen and nitrogen species [3]; this induces oxidative stress, thus stimulating chemotactic activity and inflammatory cell infiltration [2,4]. Oxidative damage reaches adjacent tissues such as haematopoietic tissue, and is related to the intensity of the inflammatory response [5]. In this phase, therapeutic interventions should

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attenuate damage in healthy myofibrils, limiting the area affected by haematoma and excessive inflammation [2]. The primary goal of rehabilitation is an earlier return to physical and/or sporting activities [1], and non-pharmacological interventions such as cryotherapy and pulsed ultrasound therapy (PUT) are often used during the inflammatory phase of muscle injury.

Cryotherapy is defined as the therapeutic application of any substance that removes heat from the body, thus lowering the temperature of tissues [6]. Immersion in cold water ($\leq$15 °C) is a common method of heat reduction [7]. Its therapeutic effects include analgesia, vasoconstriction, reduction in blood flow, and decreased metabolism, inflammation and pain [6,7]. Its application is indicated immediately after musculoskeletal injuries [8] as it attenuates microvascular dysfunction and decreases muscle and blood oxidative damage [5], leading to lower adhesion and infiltration of leukocytes in tissue [2].

Therapeutic ultrasound parameters traditionally vary in terms of application time (3 to 10 minutes), frequency (1 to 3 MHz), intensity (0.1 to 0.5 W/cm^{2SATA}) and waveform (continuous and pulsed) [4,9]. Ultrasound is usually applied in a pulsed waveform 24 hours after musculoskeletal injury [4,10] because it increases the synthesis of contractile proteins derived from satellite cells of myofibrils [11], and stimulates the alignment of collagen fibres, favouring the formation of new muscle fibres and reducing the injured area [12,13]. PUT also reduces muscle lipoperoxidation [4] and decreases the plasma concentration of neutrophils, monocytes and lymphocytes [14].

Regarding skeletal muscle injuries, previous studies have only described the isolated effects of cryotherapy [2,5,8] or PUT [4,10,12–14]. Little scientific evidence exists regarding the benefits of the use of cryotherapy and PUT in combination. As such, the aim of this study was to evaluate the effects of cryotherapy and PUT, applied individually and in combination, on oxidative stress parameters, tissue damage markers and systemic inflammation after musculoskeletal injury.

Methods

Animals and experimental groups

Animal manipulation was performed in accordance with the animal testing guide (in agreement with Brazilian Legislation N. 11.794/2008). All procedures outlined in this study were approved by the Ethics Commission on Animal Use of the Federal University of Rio Grande (No. P034/2012). Adult male Wistar rats (weight 300 to 400 g) were obtained from the central animal house of the study institution. The animals were housed at a density of three per cage with free access to food and water. All animals were maintained on a 12-hour dark/light cycle at 20 to 24 °C. Seventy animals were initially anaesthetised and divided at random, using a computer programme (www.random.org), into five homogeneous groups ($n = 14$ per group): control (animals were handled but without muscle injury or intervention); lesion (muscle injury without treatment); cryotherapy (muscle injury treated with cryotherapy); PUT (muscle injury treated with low-intensity pulsed ultrasound); and cryotherapy + PUT (muscle injury treated with cryotherapy and PUT). All treatments were repeated every 8 hours and the complete experimental protocol lasted 72 hours. Eight hours after the last treatment round, all animals were killed by decapitation, and injured gastrocnemius muscle and blood samples were collected and frozen at −80 °C for later analysis.

Muscle injury

The gastrocnemius muscle from the right calf was injured by mechanical crushing [10,15]. Animals were anaesthetised with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (15 mg/kg) before the procedure. Before injury, all animals were depilated. Animals were positioned in ventral decubitus at the base of the lesion-generating equipment with the knee fully extended and the ankle in neutral position (90°). Briefly, gastrocnemius injury was produced by a metal weight (0.2 kg) that fell through a metal guide from a 30-cm height. The kinetic energy delivered during each impact was 0.484 J and two impacts were performed. Rats in the control group were also anaesthetised and manipulated to ensure standardisation but without muscle trauma. At the end of the study, the lesion site was dissected and no signs of bone fracture were found.

Cryotherapy application

Immediately after muscle injury, animals in the cryotherapy group were immersed up to the waist in cold water (10 °C) for 20 minutes. Animals in the control and lesion groups were immersed up to the waist in water at 30 °C [16]. This temperature is considered to be therapeutically inert [17]. The water temperature was controlled by exchanging water and/or by the addition of crushed ice or hot water.

PUT application

Ultrasound equipment (Model 6763, Ibramed, Amparo, SP, Brazil) was calibrated by the manufacturer before and after the study, ensuring the linearity of the scale with the radiant force method. Pulsed ultrasound was applied at a frequency of 1 MHz and a low intensity of 0.4 W/cm^{2SPTA} (spatial peak-temporal average) for 5 minutes, using a 5-cm diameter head (N. TR3CCE02) with an effective radiating area of 3.5 cm^2. The protocol used a 20% duty cycle (2 ms on, 8 ms off) corresponding to 0.08 W/cm^{2SATA} (spatial-averaged temporal intensity) [14,18,19]. Treatment with PUT was initiated 24 hours after muscle injury, and was applied underwater (water at 30 °C).