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● *Original Contribution*

EFFECTS OF PHONOPHORESIS AND GOLD NANOPARTICLES IN EXPERIMENTAL MODEL OF MUSCLE OVERUSE: ROLE OF OXIDATIVE STRESS

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Abstract—The aim of the study described here was to investigate the effects of pulsed ultrasound and gold nanoparticles (AuNPs) on behavioral, inflammatory and oxidative stress parameters in an experimental model of overuse. Wistar rats performed 21 d of exercise on a treadmill at different intensities and were exposed to ultrasound in the presence or absence of AuNPs. The overuse model promoted behavioral changes and increased creatine kinase, superoxide dismutase and glutathione peroxidase activity, as well as the levels of superoxide, nitrotyrosine, nitric oxide, thiobarbituric acid reactive substance, carbonyl, tumor necrosis factor α and interleukin-6. These values were significantly decreased by AuNPs and by AuNPs plus ultrasound. Catalase activity remained unchanged and the glutathione level increased significantly after exposure to AuNPs plus ultrasound. These results suggest a susceptibility to anxiety as well as elevated levels of oxidative stress. However, therapeutic interventions with AuNPs plus ultrasound reduced the production of oxidants and oxidative damage and improved the antioxidant defense system. (E-mail: ap@unesc.net) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Overuse, Muscle damage, Ultrasound, Gold nanoparticles, Oxidative stress, Exercise.**

INTRODUCTION

Muscle damage caused by sports is frequent and results from the hyperextension or forced contraction of a limb or by direct contusion, and such damage is also largely due to the wearing of the skeletal muscles (Torres et al. 2007). The primary origin of the lesions may be associated with excessive use of skeletal muscle or overuse from repetitive submaximal loading of the musculoskeletal system when rest is either inadequate or does not enable structural adaptation to take place (DiFiori et al. 2014). Higher training volumes have consistently been found to increase the risk of overuse injury in multiple sports. Other factors may contribute to overuse injury, such as multiple competitive events on the same day or over several consecutive days. This factor may be better considered a marker for a high ratio of workload to recovery time (DiFiori et al. 2014).

The consequences of overuse are not clearly established and are still inconclusive, but it is possible that biochemical, molecular and behavioral changes will be among the most common changes in athletes in situations of overuse. Limited data on overuse injuries exist, most likely because of the difficulty in diagnosing pain caused by repetitive microtrauma, which often goes unreported or is overlooked by the athlete during the initial progression of the injury (Paterno et al. 2013). In addition, many athletes ignore minor pains because they are subtle and minimally affect muscle function in the initial stages (Caine et al. 2006).

Exercise-induced muscle damage frequently occurs after intense or exhaustive exercise in acute or repetitive sessions, particularly if the exercise involves eccentric muscle contractions resulting in the production of several inflammatory and biochemical mediators (Silva et al. 2011). Moreover, it is clinically expressed by a relevant set of indirect markers of muscle damage such as muscle

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soreness, decreased maximal voluntary muscle contractions and increased muscle stiffness with a reduced range of motion (Janecki et al. 2011; Torres et al. 2007). In recent years, studies have reported that reactive oxygen species (ROS) and consequent oxidative stress are directly involved in the muscle damage induced by trauma or intense physical exercise, as the injured muscle tissue stimulates generation of ROS in several cellular sites, the primary mechanism being inflammation (Bar-Shai et al. 2008; Silva et al. 2011, 2013; Silveira et al. 2012; Supinski and Callahan 2007). Therefore, the extent of the inflammatory response may determine the efficacy of muscle repair as well as the oxidative stress level (Silveira et al. 2010). Thus, it is possible that previous interventions of chronic or acute inflammation may attenuate the deleterious effects induced by muscle injury or overuse.

Therapeutic ultrasound is one of the most frequently used treatment modalities for a variety of skeletal muscle injuries (Warden and McMeeken 2002). Specifically, with respect to the effectiveness of ultrasound for treating muscular injuries, it has been found that therapeutic pulsed ultrasound (TPU) enhances myogenic precursor cell and fibroblast proliferation; it also reduces oxidative stress parameters in experimental models of muscle injury (Chan et al. 2010; Piedade et al. 2008; Silveira et al. 2013). In addition, the use of ultrasound to facilitate the delivery of transdermal or dermatologic drugs (Hauser et al. 2009; Maia Filho et al. 2010; Silveira et al. 2010) such as gold nanoparticles (AuNPs), used medically to enhance the therapeutic potential of drugs (Shukla et al. 2005), has been proposed as an anti-oxidant and anti-inflammatory agent (Victor et al. 2012).

Thus, on the basis of these assumptions, our purpose was to investigate the effects of applying therapeutic pulsed ultrasound, alone or in combination with AuNPs, on oxidative stress and inflammatory parameters in an experimental model of overuse.

METHODS

Animals

Male Wistar rats (250–300 g) were obtained from our breeding colony. The animals were housed five to a cage, on a 12-h light/dark cycle (lights on at 07:00), with free access to food (Nuvilab CR1, Nuvital Nutrientes, Colombo, Brazil) and water. All experimental procedures were performed in accordance with the Brazilian Guidelines for the Care and Use of Animals for Scientific and Didactic Purposes (DOU 27/5/13, MCTI, p. 7) and were approved by the local ethics committee. The animals were randomly divided into five groups: control (C, n = 12); exercise-overuse (EO, n = 6); exercise-overuse plus AuNPs (EO-N, n = 6); exercise-overuse plus ultrasound and saline (EO-U, n = 6); and exercise-overuse plus ultrasound and AuNPs (EO-UN, n = 6).

Overuse model

All animals were habituated on a nine-channel, motor-driven treadmill at the speed of 10 m/min for 10 min/d for 1 wk to reduce the stress of a new environment. The mice did not receive any stimuli to run. The exercise program consisted of three different models: low intensity (60 min at 13 m/min, no incline), moderate intensity (60 min at 17 m/min, no incline) and high intensity (inclination −16% until exhaustion at 17 m/min—eccentric exercise). Exhaustion was considered the inability of the animals to maintain a continuous rhythm (≥30 s at the end of the run). Exercise sessions were performed for 21 consecutive days, and one exercise model was applied daily in the following sequence: low intensity, moderate intensity and high intensity.

Treatment

Therapeutic pulsed ultrasound treatment (Imbramed, Amparo, São Paulo, Brazil) was administered in the presence or absence of AuNPs in all animals 1 h before the exercise session on day 4. The application parameters were: duration of 6 min, frequency of 1.0 MHz, intensity of 0.8 W/cm^2, effective radiating area of 1 cm^2, 50% duty cycle of 1:2 (5 ms on, 5 ms off) and a focused geometry for the ultrasound beam. The ultrasound-treated area was approximately 2 cm^2, and the movement of the beam was circular. The parameters used for TPU were established on the basis of previous results already published by our group (Freitas et al. 2007, 2010; Silveira et al. 2010).

Synthesis and characterization of AuNPs

Gold nanoparticles were prepared as described elsewhere (Victor et al. 2012). Sodium citrate and hydrogen tetrachloroaurate ($HAuCl_4$) solutions (Sigma–Aldrich, St. Louis, MO, USA) were used as received. The electronic spectrum was obtained with a Shimadzu Model UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan), and exhibited a surface plasmon resonance band with a λ_{max} at 520 nm, which is typical of spherical gold nanoparticles. X-Ray diffraction analysis was performed using a Shimadzu LAB-X Model XDR–6000 diffractometer with Cu$K\alpha$ radiation (λ = 1.54056 Á, 30 kV, 30 mA). The scan rate was 2°/min and ranged from 20 to 80°. A mean particle diameter of 25 nm was calculated using Scherer's equation (Dorset 1998). The Au concentration in the AuNPs solution was determined by atomic absorption analysis (Rocha et al. 2001; Singh et al. 2009; Sonavane et al. 2008) in a Varian Model AA 240 Z atomic absorption spectrometer (Varian Medical Systems, Palo Alto, CA, USA). A 36 mg L^{-1} solution