Ultrasound in Med. & Biol., Vol. 41, No. 3, pp. 797–805, 2015

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2014.11.017

● *Original Contribution*

ULTRASOUND MODULATES SKELETAL MUSCLE CYTOKINE LEVELS IN RATS WITH HEART FAILURE

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(*Received* 22 *February* 2014; *revised* 11 *November* 2014; *in final form* 24 *November* 2014)

Abstract—Heart failure is a multisystemic disorder that leads to an imbalance between pro- and anti-inflammatory cytokines. Therapeutic ultrasound (TU) has been reported to modulate the inflammatory process. The aim of this study was to evaluate the effect of TU on pro- and anti-inflammatory cytokine levels in soleus muscle and plasma of rats with heart failure. Thirty male Wistar rats (230–260 g) were submitted to ligation of the left coronary artery or sham surgery. Six weeks after surgery, TU was administered directly to the right lower limb. The results indicate that TU promotes reduction of pro-inflammatory cytokine levels (tumor necrosis factor α, interleukin-6) and increases anti-inflammatory cytokine levels (interleukin-10) in the soleus muscle of rats with heart failure. This is the first study to find that TU can modulate cytokine levels in rats with heart failure. Additionally, this is a first report that TU can modulate interleukin-10 levels in the soleus muscle. (E-mail: Roplentz@yahoo.com.br) **© 2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Heart failure, Therapeutic ultrasound, Cytokines, Skeletal muscle.**

INTRODUCTION

Heart failure (HF) is a multisystemic disorder the effects of which extend beyond the cardiovascular, musculoskeletal (Georgiadou and Adamopoulos 2012) and immunologic (Hasper et al. 1998; Toth et al. 2006) systems. The severity of left ventricular dysfunction reflects a series of physiopathologic changes, such as decreased oxidative capacity (Delp et al. 1997), morphologic and biochemical alterations of the muscle fibers (Georgiadou and Adamopoulos 2012), endothelial dysfunction (Ferrari et al. 1998) and activation of the neurohumoral and immunologic systems (Hedayat et al. 2010). The last is a determinant of increased muscular and plasma levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines, characteristic of this syndrome (Hedayat et al. 2010; Kaur et al. 2006).

Different mechanisms contribute to exercise intolerance symptoms in heart failure. The "muscle hypothesis" proposes that skeletal muscles play a key role in this context, where exertional dyspnea and fatigue are the results of skeletal muscle disorders (Piepoli et al. 2010). The imbalance between pro- and anti-inflammatory cytokines plays an important role in the development and progression of heart failure (Conraads et al. 2002). Increased levels of pro-inflammatory cytokines, mainly tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6), have been strongly related to structural and functional alterations in skeletal muscle, early fatigue, muscle mass loss and exercise intolerance (Larsen et al. 2002; Lunde et al. 2001; Schulze et al. 2003; Toth et al. 2006). In addition, some studies report that increased TNF-α and IL-6 levels may be important indicators of poor prognosis (Deswal et al. 2001), because these are directly related to disease severity in patients with HF (Mommersteeg et al. 2010).

In this context, inflammation emerges as an important therapeutic target in HF patients (Celis et al. 2008; Heymans et al. 2009), and some non-pharmacologic therapies have been proposed as a way to regulate the inflammatory imbalance generated in HF, for example, physical

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exercise (Nunes et al. 2008; Smart and Steele 2011) and electrical stimulation (Karavidas et al. 2006). Another therapy that has beneficial effects on the inflammatory response is therapeutic ultrasound (TU) (Nagata et al. 2013; Signori et al. 2011). TU was found to modulate various biological processes in tissue cultures and animal models; for example, it decreased concentrations of the pro-inflammatory cytokines TNF-α and IL-6 *in vitro* (Li et al. 2003). Really, therapeutic ultrasound enhanced angiogenesis and production vascular endothelial growth factor (Reher et al. 1999; Young and Dyson 1990), vasodilation (Maruo et al. 2004) and nitric oxide (NO) generation by endothelial shear stress caused by ultrasonication (Iida et al. 2006). Furthermore, TU therapy protects muscle against oxidative stress (Freitas et al. 2007) and improves the state of ischemia–reperfusion (Bertuglia 2007).

In recent years, some studies have reported the effectiveness of TU in the management of the inflammatory process (Nakamura et al. 2011; Signori et al. 2011). However, despite the positive effects of TU on the acute inflammatory profile, no studies have evaluated the effect of TU on the inflammatory process in a chronic heart failure model. We hypothesized that TU can modulate the pro-inflammatory response in skeletal muscle of rats with HF. Therefore, the aim of this study was to evaluate the effect of TU on levels of TNF-α, IL-6 and IL-10 in the soleus muscle and plasma of rats with HF.

METHODS

Experimental procedure

Studies were performed on 30 male Wistar rats (230–260 g) from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). The rats were housed in groups of three per cage and received food and water *ad libitum* in an animal room maintained at 22°C under a 12:12-h light-dark cycle. The investigation followed the ethics guidelines established by the *Guide for Care and Use of Experimental Animals* published by the U.S. National Institutes of Health, Bethesda, Maryland, USA (NIH Publication No. 85-23, revised in 1996) and in adherence with good clinical practice guidelines. All procedures outlined in this study were approved by the UFCSPA Ethics and Research Committee (Protocol 004/10).

Surgery to induce myocardial infarction and experimental groups

Rats (n = 38) were divided at random into the Plac-Sham (n = 8), TU-Sham (n = 8), Plac-HF (n = 11) and TU-HF (n = 11) groups, anesthetized with xylazine (12 mg/kg ip) and ketamine (90 mg/kg ip), endotracheally intubated (14G endotracheal tube) and artificially ventilated (Samay VR 15, Universidad de la República, Montevideo, Uruguay) at a respiratory frequency of 60 breaths/min and an oxygen inspired fraction of 100%. Myocardial infarction was induced according to the method of Pfeffer et al. (1979), which is the method usually used by our research group (Hentschke et al. 2013; Jaenisch et al. 2011; Nunes et al. 2008). The heart was exposed through left thoracotomy between the fourth and fifth ribs. For animals in which myocardial infarction was induced, a 6-O mononylon suture (Ethilon, Ethicon, São Paulo, Brazil) was passed into the main left descending coronary artery, at a point between 1 and 2 mm distal to the edge of the left atrium, and the left coronary artery was ligated. Sham-operated animals underwent the same procedure without tying the suture and served as control rats. The thorax was closed, the skin was sutured and the pneumothorax was drained by a continuous aspiration system. During the first 48 h, the animals were treated for post-operative pain with Torbugesic (0.5 mg/kg) and given a single dose of penicillin (20,000 U, ip). After myocardial infarction, the animals were allowed 6 wk for recovery (time necessary to develop the HF state) and assigned to one of four experimental groups: placebo-treated, sham-operated rats (Plac-Sham, n = 8); therapeutic ultrasound-treated, sham-operated rats (TU-Sham, n = 8); placebo-treated rats in which HF had been induced (Plac-HF, n = 7); or therapeutic ultrasound-treated rats in which HF had been induced (TU-HF, n = 7).

Treatment with therapeutic ultrasound

After 42 d, rats in the TU groups were treated with ultrasound (Sonopulse Compact, Ibramed, Amparo, SP, Brazil) administered only once directly in the right lower limb. A commercially available ultrasound gel was used as coupling agent, and all animals were depilated before the application. Ultrasound equipment was calibrated by the Biocare Medical System (Passo Fundo, RS, Brazil) before the study, ensuring linearity of scale, with the radiance force method. TU was administered for 2 min at a frequency of 1 MHz, pulse of 100 Hz and intensity of 0.4 W/cm^2 using a 3-cm-diameter head with an effective radiating area of 3.5 cm^2. The protocol comprised 2 ms on and 8 ms off, with other parameters maintained constant, which elicits a spatial averaged temporal intensity of 0.08 W/cm^2. The control animals (placebo) were manipulated in the same way but with the ultrasound equipment turned off. The methodology and TU parameters were adapted from previous studies conducted by our group (Signori et al. 2011).

Cardiac hemodynamic assessment

Immediately after TU, animals were anesthetized as previously described, and the skin was shaved using a