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● *Original Contribution*

EFFECTS OF DIFFERENT THERAPEUTIC ULTRASOUND WAVEFORMS ON ENDOTHELIAL FUNCTION IN HEALTHY VOLUNTEERS: A RANDOMIZED CLINICAL TRIAL

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Abstract—The purpose of this study was to determine the effects of different therapeutic 1-MHz ultrasound waveforms on endothelial function before and after cyclooxygenase (COX) inhibition. Forty-two healthy volunteers aged 27.2 ± 3.8 y underwent interventions and an evaluation for endothelial function (n = 15; with COX inhibition, n = 15; duration of the vasodilator effect, n = 12) by technique flow-mediated dilation. Continuous ultrasound therapy (0.4 W/cm$^{2\ SATA}$), pulsed ultrasound therapy (20% duty cycle, 0.08 W/cm$^{2\ SATA}$) or placebo (equipment power off) was randomly applied over the brachial artery for 5 min. COX inhibition (aspirin) was carried out 30 min before treatments. In relation to the placebo, flow-mediated dilation increased by 4.8% using continuous ultrasound and by 3.4% using pulsed ultrasound. After COX, flow-mediated dilation was enhanced by 2.1% by continuous ultrasound and 2.6% by pulsed ultrasound. This vasodilation persisted for 20 min. Continuous and pulsed therapeutic 1-MHz ultrasound waveforms improved endothelial function in humans, which provided them with anti-inflammatory vascular effects. (E-mail: l.signori@hotmail.com) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Endothelial function, Vascular endothelium, Ultrasonic therapy, Ultrasound, Cyclooxygenase inhibitors, Nitric oxide.**

INTRODUCTION

The vascular endothelium plays a crucial role in hemostatic balance and vascular health through its response to physical or chemical stimuli, as well as through the production and/or release of vasoactive molecules (Vanhoutte et al. 2009). Nitric oxide (NO) is the universal orchestrator responsible for the regulation of vascular tone, inhibition of platelets and leukocyte aggregation, suppression of smooth muscle cell proliferation and stimulation of angiogenesis in conjunction with prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF) (Higashi et al. 2009; Poredos and Jezovnik 2013; Rajendran et al. 2013; Vanhoutte et al. 2009). In an inflammatory response, an increase occurs in reactive oxygen species (ROS), mainly the superoxide anion radical ($O_2\bullet^-$), which can react and reduce the bioavailability of NO by forming peroxynitrite. This reaction inhibits the enzymatic activity of prostacyclin synthase and stimulates the production of other eicosanoids (Gryglewski 2008; Vanhoutte et al. 2009). This action of ROS on the endothelial monolayer may establish a vicious circle that results in inflammation and endothelial dysfunction (Deanfield et al. 2007).

In humans, the action of prostaglandin H synthase (or cyclooxygenases [constitutive COX-1 and inducible COX-2]) on arachidonic acid is also responsible for the synthesis of eicosanoids that are found in inflamed

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tissues (Gryglewski 2008; Vanhoutte et al. 2009). The production of PGI_2 in the vascular endothelium is COX-2 dependent and contributes to vascular homeostasis at appropriate concentrations (Gryglewski 2008). However, the increased production of inflammatory cytokines, which modify platelet aggregation (Buerkle et al. 2004) and the adhesion of leukocytes (Vanhoutte et al. 2009), favors the formation of other isoforms of prostaglandins (PGE_2, PGF_{2a}, PGD_2), particularly thromboxane (TxA_2), which has vasoconstrictor action (Gryglewski 2008; Vanhoutte et al. 2009). Clinically, endothelial function measured by technique flow-mediated dilation (FMD) is a strong predictor of cardiovascular events (Poredos and Jezovnik 2013; Shechter et al. 2014; Thijssen et al. 2011; Xu et al. 2014) and all-cause mortality (Xu et al. 2014).

"Low-power" therapeutic ultrasound is broadly used in physical therapy, mainly in the treatment of soft tissue injuries. Its therapeutic effects are dependent on its application parameters, such as frequency (1–3 MHz), intensity (0.1–3 W/cm$^{2\ SATA}$), application time (1–10 min) and type of wave (continuous or pulsed) (Baker et al. 2001; O'Brien 2007). A portion of the incident field is absorbed by endothelial cells and blood, while the ultrasonic wave propagates throughout the tissue, producing localized effects, such as increased shear stress (Bertuglia 2007) and formation of a stable microbubble (Juffermans et al. 2009; ter Haar 2007). Experimental studies have indicated that low-intensity ultrasound promotes vascular dilation (Altland et al. 2004; Bertuglia 2007; Sugita et al. 2008), increases the level of endothelial NO (Sugita et al. 2008), decreases COX-2 expression (Nakamura et al. 2011), reduces the formation of ROS (Bertuglia 2007; Juffermans et al. 2009) and induces neoangiogenesis (Ramli et al. 2009). Clinically, low-intensity pulsed (0.12 W/cm$^{2\ SATA}$, 29 kHz, 30% duty cycle) ultrasound dilates human brachial arteries (Iida et al. 2006). However, physiotherapists use the ultrasonic equipment with different parameters, mainly in relation to the frequency (MHz) and waveforms (continuous and pulsed), the effects of which on endothelial function have yet to be determined in humans.

Different types of low-frequency therapeutic ultrasound waves (27 kHz, 0.25 W/cm$^{2\ SATA}$) may generate distinct outcomes, such as an increase in NO production by endothelial cells; the pulsed (10% duty cycle) waveform particularly appears to be more effective (Altland et al. 2004). In muscle injury in rats, the continuous waveform (1 MHz, 0.4 W/cm$^{2\ SATA}$) promotes a reduction in erythrocytes and an increase in segmented neutrophils in the blood (Plentz et al. 2008). Furthermore, the pulsed waveform (1 MHz, 20% duty cycle, 0.08 W/cm$^{2\ SATA}$) reduces the total leukocyte count as a result of the lower concentrations of segmented neutrophils, monocytes and lymphocytes, without altering the level of circulating erythrocytes (Signori et al. 2011). These effects on acute muscle inflammation may influence muscle regeneration (Signori et al. 2014), because only the pulsed waveform decreases muscle damage after 7 d of treatment (Fisher et al. 2003). Because endothelial and blood cells interact directly with ultrasonic waves and high-frequency devices have not yet been tested in humans, we hypothesized that different 1-MHz ultrasound waveforms may promote distinct endothelium-dependent vasodilation outcomes in relation to the production of NO and PGI_2. The aims of our study were to compare the effects of continuous and pulsed therapeutic ultrasound waveforms on endothelial function and to establish the duration of these effects and identify the pathway responsible for the vascular response.

METHODS

Design overview

This controlled, randomly assigned, crossover, double-blind, three-arm therapeutic trial was approved by the Health Research Ethics Committee at the Universidade Federal do Rio Grande (CEPAS-FURG, No. 88/2012) and was registered in the Brazilian Clinical Trials Registry (Protocol RBR-4 z5 z3 t). The evaluations were carried out at the Imaging Centre of the University Hospital of Dr. Miguel Riet Corrêa, Jr. The methodologic design was based on the determinations of the 2010 CONSORT statement. Patients were informed of the study protocol and provided written informed consent before participating.

Setting and participants

All enrolled patients were alphabetized volunteers aged 20 to 35 y, with a body mass index (BMI) lower than 30 kg/m^2; non-smokers; and free of skeletal muscle, rheumatic, cardiovascular, metabolic, neurologic, oncologic, immune, hematologic, psychiatric or cognitive disorders. The enrolled patients were not taking any type of medication. Individuals with an inflammatory response (C-reactive protein $>$3 mg/dL or fibrinogen $<$200 or $>$400 mg/dL), leukocytosis (11.000 $\times$ 10^3/mm^3), alcohol intake within 24 h before evaluations, arterial diameters $<$2.5 mm or $>$5.0 mm and endothelial dysfunction assessed by flow-mediated dilation (FMD $<$8%) were excluded.

Outcomes and follow-up

The main outcome was endothelial function, as measured by FMD (%). The secondary outcome was endothelium-independent vasodilation, as evaluated by the vascular response to nitroglycerin and the duration