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● *Original Contribution*

EFFECT OF LOW-INTENSITY CAVITATION ON THE ISOLATED HUMAN THORACIC ARTERY *IN VITRO*

ALGIMANTAS BUBULIS,* VIDA GARALIENĖ,* VYTAUTAS JURĖNAS,* JONAS NAVICKAS,* and SAULIUS GIEDRAITIS†

*Kaunas University of Technology, Kaunas, Lithuania; and †Lithuanian University of Health Sciences, Kaunas, Lithuania

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Abstract—Reported here are the results of an experimental study on the response to low-intensity cavitation induced by low-frequency (4–6 W/cm^2, 20 kHz and 32.6 kHz) ultrasound of isolated human arterial samples taken during conventional myocardial revascularization operations. Studies have found that low-frequency ultrasound results in a significant (48%–54%) increase in isometric contraction force and does not depend on the number of exposures (10 or 20) or the time passed since the start of ultrasound (0, 10 and 20 min), but does depend on the frequency and location (internal or external) of the blood vessels for the application of ultrasound. Diltiazem (an inhibitor of slow calcium channels) and carbachol (an agonist of muscarinic receptors) used in a concentration-dependent manner did not modify the relaxation dynamics of smooth muscle affected by ultrasound. Thus, ultrasound conditioned to the augmentation of the isometric contraction force the smooth muscle of blood vessels and did not improve endothelial- and calcium channel blocker-dependent relaxation. (E-mail: Vida.garaliene@gmail.com) **© 2017 The Authors. Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).**

Key Words: **Ultrasound, Human arteries, Contraction force, Diltiazem, Carbachol.**

INTRODUCTION

Ultrasound (US) has been developed for two main purposes in medicine: (i) as a valuable diagnostic tool and (ii) as a powerful stimulant of various biological effects that could be useful for the treatment of many types of diseases (Bubulis et al. 2011, 2013). These effects might be conditioned by mechanical vibrations of circulating microbubbles that are able to (i) cause stress in nearby tissues; (ii) directly trigger transient effects, which modulate the penetration through the cell membrane of various drugs (*e.g.*, penetration into vascular tissue of drugs that dissolve blood clots); (iii) direct the drugs to optimal locations for their distribution through the body (Ashokkumar 2011; Sutton et al. 2013). US, in passing through liquid, causes mechanical vibration of the fluid's molecules; if this fluid contains dissolved gas nuclei, which will be the case under normal conditions, then they are able to grow and collapse (Kimmel 2006). Acoustic cavitation is the ultrasound phenomenon by which the microbubbles oscillate and collapse (Ashokkumar et al. 2007). When cavitation bubbles oscillate and collapse, there are several physical effects, such as shock waves, microflows and turbulence. Acoustic cavitation has been found to be valuable in diagnostic and therapeutic medicine (Cavalieri et al. 2010; Hassan et al. 2010). Despite the use of ultrasound in a variety of applications (Ahmadi et al. 2012; Choi et al. 2014; Miller et al. 2014), studies aimed at gaining a fundamental understanding of the mechanisms underlying the interaction of ultrasound and biologic tissue are limited. Experimental studies have indicated several bio-effects mediated by interactions between ultrasound, microbubbles and tissue cell membranes (Ahmadi et al. 2012; Ma et al. 2013; Wu and Nyborg 2008). According to Juffermans et al. (2006), these effects can be divided into three groups: thermal effects, chemical effects and mechanical effects.

In recent years, series of tests have been adopted for the evaluation of blood vessel endothelial function,

Address correspondence to: Vida Garalienė, Kaunas University of Technology, Studentu str. 56, Kaunas, Lithuania. E-mail: Vida.garaliene@gmail.com

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because studies have indicated that endothelial dysfunction can be interpreted as an independent risk factor in patients with suspected coronary insufficiency (Landmesser et al. 2004; Monnink et al. 2004). A damaged endothelium is not able to release endothelium-dependent vascular smooth muscle relaxing factors; therefore, it is still necessary to explore various tools that can potentially restore endothelial function. Ultrasound is now also widely applied in diagnosis of circulatory system disorders, but there is very little research on the impact of the field of ultrasound on physiologic functions of endothelium and smooth muscles associated with vessel contraction and relaxation (Bonow et al. 2016).

The goal of the present study was to investigate the response of isolated human arterial samples to low-intensity cavitation of low-frequency (4-6 W/cm^2, 20 kHz and 32.6 kHz) ultrasound. We hypothesized that ultrasound with the aforementioned parameters would modulate the vascular effects related to contraction and relaxation processes. An ultrasonic processor (Model VCX 130 PB, Sonics & Materials, Newtown, CT, USA) with a probe tip diameter of 3 mm was used for ultrasonic treatment of the arterial samples.

Present studies have shown that by the action of low-intensity cavitation of low-frequency ultrasound conditioned to augment the isometric contraction force of the smooth muscle of blood vessels did not improve the endothelial and calcium channel blocker-dependent relaxation.

METHODS

Experiments were carried out on isolated human thoracic artery samples obtained during conventional myocardial revascularizations from patients who underwent coronary artery bypass grafting in the Department of Cardiothoracic and Vascular Surgery at the Lithuanian University of Health Sciences. All patients signed a letter of informed consent, and the study was approved by the Regional Ethics Committee of Biomedical Research on 05/11/2010 under License No. BE-2-64, in Kaunas, Lithuania.

Arterial samples were obtained from 152 patients whose average age was 67.3 ± 9.6 y; 30.7% were women.

Vascular preparation

For investigation of blood vessel segments *in vitro,* a tissue/organ bath system purchased from Global Town Microtechnology (Sarasota, FL, USA) was used. At room temperature, arterial samples were gently cleansed of connective tissue; cut into 3- to 4-mm-long rings; hung on a vascular holder, the upper hook of which was attached to an isometric force transducer; and dipped in 5-mL tissue baths filled with Tyrode's solution warmed to 37°C and continuously bubbled with 100% O_2. The solution had the following composition (in mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.9; Tris HCl, 10; and glucose, 5; pH 7.4. Blood vessel samples were allowed to equilibrate for at least 45–60 min before the start of the experiments. During the investigation period, the preparations were washed every 15 min with fresh Tyrode's solution.

To examine the impact of the ultrasound, the side and the open end of the blood vessel were irradiated using the contraction–relaxation process, and after an equilibration period, the isolated vascular samples were subjected to 10- or 20-s discontinuous ultrasound pulses. The tip of the transducer was positioned 0.5 cm from the sample surface or along the vessel lumen.

Protocol

In the first series of experiments, we studied the isometric contraction and relaxation effects on arterial rings after a phenylephrine-induced contraction produced 0, 10 and 20 min after a 10-s ultrasound pulse. In all experiments, phenylephrine was used at a concentration of 10^{-4} M. As a vasoconstrictor, phenylephrine binds to α_1-adrenoceptors located on the surface of the sarcolemma and activates the enzyme phospholipase C, which is responsible for the synthesis of inositol 1,4,5-triphosphate (IP_3). The latter affects inositol 1,4,5-triphosphate receptors (IP_3Rs) that are localized on the surface of the sarcoplasmic reticulum (SR) in vascular smooth muscle and that dominate there (Marchant and Taylor 1997). At the same time, the sarcolemma depolarizes, and external calcium enters the cell through L-type Ca^{2+} channels. Because of the interaction between IP_3 and IP_3 receptors, the calcium ions entering the cytosol initiate Ca^{2+} release from the SR (Sipido et al. 1995). The greater the number of Ca^{2+} ions that accumulate in the SR, the greater the number that should move into the cytoplasm during depolarization; therefore, the isometric contraction of smooth muscle should increase proportionally (Bers 2002; Earley and Nelson 2006; Webb 2003).

The influence of ultrasound on the function of the endothelium or slow (L-type) calcium channels in the vessel rings was evaluated by investigating the relaxation response to carbachol, an agonist of muscarinic acetylcholine receptors (Bolton et al. 1984; Mitsui and Karaki 1990) and diltiazem, an inhibitor of slow calcium channels (van Breemen et al. 1981, 1982). When the phenylephrine-induced isometric contraction reached its steady state, the aforementioned agents were added to the solution in a cumulative concentration fashion at doses of 10^{-7} to 10^{-4} M. The time course of force was recorded. The relaxation and isometric contraction forces