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● *Original Contribution*

MATURATION OF LESIONS INDUCED BY MYOCARDIAL CAVITATION-ENABLED THERAPY

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Abstract—Myocardial contrast echocardiography at enhanced therapeutic parameters may be a novel means of tissue reduction therapy, as for hypertrophic cardiomyopathy. Dahl/SS rats were anesthetized and treated with high-amplitude pulsed ultrasound guided by 10-MHz ultrasound images. Contrast microbubbles were infused *via* the tail vein during intermittent pulse-burst exposure at 4 MPa. A sham group, a low-impact group (group A, 5 cycle pulses with Gaussian modulation and 1:4 trigger for 5 min) and a high-impact group (group B, 10 cycle pulses with 4-ms square modulation and 1:8 trigger for 10 min) were tested. The higher exposure used in group B yielded more substantial injury than the lower exposure in group A. Treated rats in both groups A and B had significant increases in wall thickness measured by echocardiography the next day, which returned to normal by the end of 6 wk. Six weeks after ultrasound exposure, heart tissue samples exhibited tissue fibrosis in Masson's trichrome stained histology. Maturation of lesions involved fibrosis replacement, preserving structural tissue integrity. This study indicates that myocardial injury noted previously progresses into permanent loss of myocardial tissue that may be sufficient for possible hypertrophic cardiomyopathy therapy. More research is needed to define the treatment parameters required for symptomatic relief for hypertrophic cardiomyopathy. (E-mail: xiaofalu@umich.edu) **© 2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound therapy, Hypertrophic cardiomyopathy treatment, Ultrasonic cavitation microlesions, Tissue reduction therapy.**

INTRODUCTION

Ultrasound contrast agents consist of suspensions of stabilized microbubbles that provide strong echogenicity when introduced into the blood vessels. The non-linear response of microbubbles provides novel opportunities for diagnostic imaging. While at the same time, the cavitation response of microbubbles to ultrasound pulses can induce bio-effects in mammalian tissue, which can be directed non-invasively for therapeutic purposes. Cavitational injury can be manipulated using different ultrasound methods for targeted cardiovascular therapy (Laing and McPherson 2009). Cavitation activity in blood has been used for thrombolysis (sonolysis) in therapy of myocardial infarction (Slikkerveer et al. 2011). Microvascular injury can be used for targeted drug delivery (Unger et al. 2014). Diagnostic myocardial contrast echocardiography (MCE) has been reported to be capable of inducing premature ventricular contraction and lethal injury of cardiomyocytes in a rat model of MCE (Miller et al. 2005). This finding indicated another potential application for directed lethal cardiomyocyte injury for the purpose of myocardial tissue reduction in hypertrophic cardiomyopathy (HCM). Hypertrophic cardiomyopathy is the most prevalent inherited cardiac disease and is estimated to affect 0.2% of the U.S. population (Maron et al. 2012; Semsarian et al. 2015). Hypertrophy can occur in several regions of the myocardium and is particularly troubling when left ventricular outflow tract (LVOT) obstructions develop. Invasive septal reduction therapy is the treatment of choice for symptomatic patients, with surgical septal myectomy being the first consideration and alcohol septal ablation an alternative (Gersh et al. 2011). However, both methods have limitations and a potential for adverse consequences; a minimally invasive method based on MCE-induced cardiomyocyte injury might provide a beneficial treatment option for some patients.

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We have proposed a novel technique using MCE and higher than diagnostic pressure amplitudes called myocardial cavitation-enabled therapy (MCET) (Miller et al. 2014a). Scattered cardiomyocyte injury was induced by the interaction of ultrasound pulses with contrast microbubbles, which may provide a relatively tolerable means of tissue reduction therapy. In this method, sites of ultrasonic cavitation nucleation lead to microlesion production in rat hearts within the ultrasonic focal zone. Treatment can be optimized by varying the timing of pulses relative to the cardiac cycle (Miller et al. 2014b) and by varying ultrasound exposure parameters to allow advantageous combination of imaging and treatment targeting (Miller et al. 2015). The targeted accumulation and distribution of myocardial necrosis have been assessed using Evan's blue staining of injured cardiomyocytes in tissue histology slides for samples taken 1 d after exposure (Zhu et al. 2015a). Unlike the infarct-like lesions induced by alcohol infusion (Baggish et al. 2006), the scattered microlesion injury appears to be adjustable from modest fractions of tissue volume to more dense distributions within a targeted focal zone. However, the longer-term maturation of the myocardial volumes with microlesions remains uncertain.

The aim of this study was to observe and characterize the maturation of the lesions and the extent of viable tissue loss 6 wk after MCET under two different ultrasound exposure parameter settings. The changes in left ventricular morphology were followed by diagnostic imaging, and changes in the electrocardiogram (ECG) were observed. The fraction of myocardial tissue destroyed and the resulting scar formation were evaluated.

METHODS

Animal preparation

All *in vivo* animal procedures were conducted with the approval and guidance of the University Committee on Use and Care of Animals of the University of Michigan. Three rats were lost from the study because of a technique problem with parameter setting in one rat and anesthetic deaths in two rats. A total of 24 male Dahl/SS rats (Charles River, Wilmington, MA, USA) weighing an average of 312 ± 19 g were included in the study. The Dahl/SS rats were used, rather than the Sprague-Dawley strain used previously, because Dahl/SS rats have been used as the base strain for a novel rat model of HCM (Kriegel and Greene 2008). The rats were anesthetized by intraperitoneal injection of a mixture of ketamine (90 mg kg^{-1}) and xylazine (9 mL kg^{-1}). The left thorax was shaved and depilated for ultrasound transmission. A 24-gauge cannula (BD Angiocath, Becton Dickinson Infusion Therapy Systems, Sandy, UT, USA) was inserted into the tail vein for intravenous injections of contrast agent. The rats were mounted on a positioning board, and ECG needle electrodes were placed in the forelegs and left hind leg. Placement of the board in a 37°C degassed and de-ionized water bath allowed for subsequent ultrasound exposures.

Ultrasound

Ultrasound exposure for treatment was provided by a laboratory system with guidance by diagnostic ultrasound imaging, as described previously (Miller et al. 2014a). Briefly, the treatment system consisted of a function generator for generating a pulse train (Model 3314 A function generator, Hewlett Packard, Palo Alto CA, USA), an arbitrary waveform generator for amplitude modulation of the pulse train (Model 33220 A, Agilent Technologies, Loveland CO, USA), a power amplifier (A-500, Electronic Navigation Industries, Rochester NY, USA) and a 1.5-MHz single-element treatment transducer (Panametrics A3464, Olympus, Waltham, MA, USA). The damped 1.9-cm-diameter treatment transducer had a 3.8-cm focal length, with a −6-dB beam diameter of 3.5 mm. The treatment was targeted with the aid of diagnostic ultrasound imaging (GE Vivid 7 with S-10 probe, GE Vingmed Ultrasound, Horten, Norway) operated at 10 MHz with 5-cm focal depth, as previously described (Miller et al. 2014a). The imaging probe and therapeutic transducer were clamped together at a 37° angle so that the therapy transducer focus and heart were co-located in the field of view of the imaging probe during treatment. For targeting, the rat heart was located at 2.25-cm depth at the edge of the sector scan, and then the transducer assembly was re-positioned to place the anterior left ventricular wall at the center of the field of view at 2.75-cm depth for exposure (Fig. 1). The vertical position was adjusted so that the beam impinged approximately at the middle of the left ventricle.

The function generator created a continuous pulse train at a 4-kHz pulse repetition frequency. The peak rarefactional pressure amplitudes (PRPAs) of the pulses were measured using a calibrated hydrophone (Model 805, Sonora Medical Systems, Longmont, CO, USA), and a 4-MPa PRPA amplitude was obtained. The estimated peak positive pressure was 10 MPa. However, the pulses were measured in water, so the positive peaks are exaggerated relative to the negative peaks. The PRPA is thought to be the most relevant parameter for cavitation, as it is, for example, used in the mechanical index. The peak positive pressures also are not particularly helpful, because they have finite amplitude distortion that makes extrapolation through the chest wall rather uncertain. The amplitude modulation was set to give zero exposure unless a modulation-envelope signal was triggered. The