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● *Original Contribution*

USE OF THERANOSTIC STRATEGIES IN MYOCARDIAL CAVITATION-ENABLED THERAPY

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Abstract—The accumulation of microlesions induced by ultrasound interaction with contrast microbubbles in the myocardium potentially represents a new method of tissue reduction therapy. Anesthetized rats were treated in a heated water bath with 1.5-MHz focused ultrasound pulses triggered once every four heartbeats from the electrocardiogram during infusion of microbubble contrast agent. Treatment was guided by an 8-MHz B-mode imaging transducer, which also was used to provide estimates of left ventricular echogenicity as a possible predictor of efficacy during treatment. Strategies to reduce prospective clinical treatment durations were tested, including pulse modulation to simulate a theranostic scanning strategy and an increased agent infusion rate over shorter durations. Sources of variability, including ultrasound path variation and venous catheter placement, also were investigated. Electrocardiographic premature complexes were monitored, and Evans-blue stained cardiomyocyte scores were obtained from frozen sections. Left ventricular echogenicity reflected variations in the infused microbubble concentration, but failed to predict efficacy. Comparison of suspensions of varied microbubble size revealed that left ventricular echogenicity was dominated by larger bubbles, whereas efficacy appeared to be dependent on smaller sizes. Simulated scanning was as effective as the normal fixed-beam treatment, and high agent infusion allowed reduced treatment duration. The success of these theranostic strategies may increase the prospects for realistic clinical translation of myocardial cavitation-enabled therapy. (E-mail: douglm@umich.edu) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Myocardial contrast echocardiography, Arrhythmia, Cardiac myocyte necrosis, Hypertrophic cardiomyopathy.**

INTRODUCTION

The ultrasonic activation of microbubbles in the circulation for therapeutic purposes continues to be explored (Aryal et al. 2014; Ibsen et al. 2013a; Sun et al. 2014; Unger et al. 2014). Ultrasound microbubble activation therapies have attractive features, including the ability to guide the treatment using ultrasound imaging and real time feedback from the microbubble emissions (Cochran et al. 2011; Kooiman et al. 2014; Vignon et al. 2013). Prospective treatments range from transient opening of capillaries, such as the blood–brain barrier (Burgess and Hynynen 2014), to complex methods for drug or gene delivery using specially prepared gaseous delivery vehicles that can be targeted to the desired region for treatment (Ma et al. 2013). These methods have been studied for treatments such as gene therapy, cancer treatment and thrombolysis (Ibsen et al. 2013b; Laing and McPherson 2009; Unger et al. 2014). However, there are significant barriers to clinical translation of laboratory methods, for example, in drug delivery (Lanza et al. 2014), and further intensive research is needed on these and other novel treatment ideas to improve prospects for translation to the clinic.

Recently we proposed the possible use of contrast agent microbubbles to produce scattered sites of ultrasonic cavitation nucleation and microlesion production in the heart, which may provide a relatively safe means of tissue reduction therapy (Miller et al. 2014a). This myocardial cavitation-enabled therapy (MCET) should allow graded treatment with ultrasound image guidance and healing with minimal scar formation. This treatment method could be beneficial in hypertrophic cardiomyopathy (HCM) and other ventricular hypertrophies. HCM is the most common

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genetic cardiovascular disease, occurring in 1 in 500 people (Maron et al. 1995). The hypertrophy can lead to asymmetric enlargement of the intraventricular septum with obstruction of the left ventricular outflow tract. Young athletes with HCM may be completely asymptomatic until vigorous exercise induces cardiac symptoms, even sudden death (Ommen and Nishimura 2004). The optimum method for diagnosis, including screening of athletes, is echocardiography, which can be used to image and characterize the hypertrophy and potential obstruction. Up to one-third of patients become candidates for myocardial reduction (Marian 2009), which is typically accomplished by septal myectomy (Maron et al. 2003). Other technologies have been studied as possible alternatives to heart surgery for HCM. Thermal ablation with high-intensity focused ultrasound (Otsuka et al. 2007), radiofrequency catheter ablation (Lawrenz et al. 2011) and transcatheter alcohol ablation (Leonardi et al. 2010) have been explored with limited success and often with added risk of arrhythmia (Marian 2009; Nishimura and Ommen 2010). A less-invasive, more tolerable alternative to surgery or total regional ablation remains elusive, but would represent a welcome treatment option.

In the MCET method, ultrasound contrast agent microbubbles are continuously infused intravenously, while intermittent contrast destruction and refill cycles are completed using focused therapy transducers, gradually building up the desired level of microlesion density. The adjustable treatment process allows for minimization of the physiologic impact on cardiac function during treatment. The end of systole has been found to be an optimal trigger time point producing premature complexes (PCs) identifiable on the electrocardiogram (ECG) and substantial cardiomyocyte injury, but minimal cardiac functional disruption (Miller et al. 2014b). The problem of optimization of the treatment caused by uncertainty in the visual scoring of microlesion accumulation after therapy-level treatment seems to be amenable to quantitative image analysis. Three-dimensional analysis of stacked tissue sections revealing microlesions provides an effective means to characterize the overall impact or "macrolesion" created in the treated volume (Zhu et al. 2015).

The aim of this study aims was to make the MCET method more amenable to clinical translation by enhancing the technique in three ways: reducing the duration of exposure to high-intensity focused ultrasound (HIFU), adding real-time control of lesion formation and providing accurate measures of microbubble dose in the myocardium.

METHODS

Approach

To achieve the first objective of reducing exposure to HIFU, a Gaussian pulse modulation envelope was tested to gauge the potential efficacy of treatment with a rapidly scanned therapy beam. Previously, 5-min exposures were used to treat a single focal spot, which would entail prohibitively long treatments even for small tissue volumes. The Gaussian modulation simulated the pulse-sequence modulation seen with a scanned beam, as in diagnostic imaging (Miller et al. 2007). The use of scanned beams for therapy treatments would be rapid and accurate in covering a target volume, combining imaging and treatment. The strategy has been called "theranostic" ultrasound (Martin and Dayton 2013) and represents a promising means of creating cavitation-enabled therapies suitable for clinical application. The desired treatment volume for hypertrophic cardiomyopathy already can be identified and mapped for surgery using 3-D ultrasound (Sadat et al. 2013). The prospective theranostic method for MCET would simply add a high-power scan region set in the image to include the volume targeted for tissue reduction.

Another requisite goal will be to control treatment progress in real time and to predict the numbers and distribution of microlesions within hypertrophied regions of the myocardium. In small animals, the real-time control of MCET involves observation of at least three possible control predictive indicators for guiding adjustment of exposure factors. One is provision of a consistent ultrasound path for the therapeutic beam to the myocardium, which can be accomplished by image guidance. Our system employs an 8-MHz sector scanner to thread a 1.5-MHz fixed beam through the "window" between two ribs and between the sternum and left lung lobe (Miller et al. 2014a). However, this is approximate, and some means to monitor the transmission losses to the heart would be of value. Another factor is the occurrence in the ECG signal of PCs, which are strongly correlated with cardiomyocyte injury (Miller et al. 2011). Previously, this easily monitored indicator was found to be useful for assessing whether or not effective conditions were active, but the relationship to the actual total effect was problematic for highly efficacious conditions (Miller et al. 2014b).

A third factor is the microbubble dose actually delivered to the myocardium. In previous research on capillary injury in kidney, the delivered dose was found to vary from animal to animal and also during the treatment because of microbubble destruction (Miller et al. 2010). For this study, we hypothesized that the microbubble-laden blood in the left ventricle provides a real-time observable indicator of circulating microbubble dose, including destruction and refill. This hypothesis was examined by analyzing images of the left ventricle to measure the echogenicity of image regions set entirely within left ventricular blood. In addition, the influence of modified microbubble suspensions with enhanced