Ultrasound in Med. & Biol., Vol. 40, No. 6, pp. 1228–1236, 2014

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2014.01.001

● *Original Contribution*

OPTIMIZATION OF ULTRASOUND PARAMETERS OF MYOCARDIAL CAVITATION MICROLESIONS FOR THERAPEUTIC APPLICATION

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(*Received* 19 *July* 2013; *revised* 25 *November* 2013; *in final form* 2 *January* 2014)

Abstract—Intermittent high intensity ultrasound scanning with contrast microbubbles can induce scattered cavitation microlesions in the myocardium, which may be of value for tissue reduction therapy. Anesthetized rats were treated in a heated water bath with 1.5 MHz focused ultrasound pulses, guided by an 8 MHz imaging transducer. The relative efficacy with 2 or 4 MPa pulses, 1:4 or 1:8 trigger intervals and 5 or 10 cycle pulses was explored in six groups. Electrocardiogram premature complexes (PCs) induced by the triggered pulse bursts were counted, and Evans blue stained cardiomyocyte scores (SCSs) were obtained. The increase from 2 to 4 MPa produced significant increases in PCs and SCSs and eliminated an anticipated decline in the rate of PC induction with time, which might hinder therapeutic efficacy. Increased intervals and pulse durations did not yield significant increases in the effects. The results suggest that cavitation microlesion production can be refined and potentially lead to a clinically robust therapeutic method. (E-mail: douglm@umich.edu) © **2014 World Federation for Ultrasound in Medicine & Biology.**

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

INTRODUCTION

Suspensions of stabilized microbubbles have been approved as commercial contrast agents for ultrasound (US) imaging. The highly non-linear response of the microbubbles provides novel opportunities for diagnostic imaging (Averkiou et al. 2003). In myocardial contrast echocardiography, contrast agents flow into the myocardial capillaries and therefore can reveal variation in myocardial perfusion (Dijkmans et al. 2006; Porter and Xie 2010). The strong, cavitation-like response of the microbubbles to even diagnostic pulses of relatively high intensity can lead to a variety of microscale bioeffects in contrast enhanced diagnostic ultrasound (DUS) (Miller et al. 2008). These cavitational bioeffects have the potential to be harnessed for therapeutic purposes. Potential cardiovascular applications presently under study include directed delivery of gases, drugs, genes, stem cells and enhanced thrombolysis (Laing and McPherson 2009). Myocardial contrast echocardiography has been shown to be capable of generating premature electrocardiographic complexes and lethal injury of cardiomyocytes in an animal model (Miller et al. 2005a). This phenomenon leads to randomly scattered microlesions involving one or a few lethally injured cardiomyocytes as seen in histology, which appear to gradually resolve with minimal scaring over about 6 wk in rats (Miller et al. 2005b). This unique phenomenon may present an attractive opportunity for development of non-invasive therapeutic applications.

The most obvious application may be for myocardial reduction therapy, which can be beneficial for hypertrophic cardiomyopathy (HCM) and other ventricular hypertrophies. HCM is the most common genetic cardiovascular disease, occurring in 1 in 500 people (Maron et al. 1995). Young athletes with HCM may be completely asymptomatic until exercise-induced shortness of breath, angina, palpitations and even sudden death is experienced (Ommen and Nishimura 2004). The hypertrophy changes and stiffens the cardiac muscle, commonly leading to asymmetric enlargement of the intraventricular septum that can cause obstruction of the left ventricular outflow tract. Echocardiography is

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the optimum method for diagnosis, potentially including routine screening of athletes, and for treatment follow-up, owing to its dynamic real-time imaging and null ionizing radiation dose. About one-third of patients remain symptomatic after pharmacologic therapy and are candidates for myocardial reduction (Marian 2009). The standard method for therapeutic reduction is septal myectomy, specifically for removing septal hypertrophy involving perturbation of the mitral valve leaflets (Maron et al. 2003).

Alternatives to heart surgery for HCM have been sought using various technologies. Thermal ablation with high intensity focused US can be used to reduce cardiac tissue by accumulating focal lesions, but has limited penetration and requires a large aperture (*i.e.,* open chest in the canine model) (Otsuka et al. 2007). Radiofrequency catheter ablation of septal hypertrophy failed to produce efficacious septal narrowing and led to a need for pacemaker implantation in 21% of patients (Lawrenz et al. 2011). Transcatheter septal ablation with alcohol injection into the coronary arteries also has been developed as an alternative to surgery with some encouraging results (Leonardi et al. 2010). However, this procedure also has about a 20% incidence of heart block requiring permanent pacemaker (Marian 2009). In addition, there is a substantial risk of serious arrhythmia arising from healing of the alcohol infarction to scar with subsequent cardiac remodeling (Nishimura and Ommen 2010). A less-invasive, safer alternative to surgery or alcohol ablation would be a welcome addition to treatment options.

Myocardial cavitation-enabled therapy (MCET), in which sufficient microlesions are accumulated for therapeutic myocardial reduction, may provide a novel means of HCM management. The fusion of established diagnostic echocardiography with this new therapeutic method should represent a relatively gentle and advantageous combination. With refinement, MCET may be an adjunct to standard treatment approaches, used primarily in patients who are too high risk for surgery, or used prophylactically to prevent significant hypertrophy and obstruction. This development could significantly improve outcomes and quality of life for patients living with this life threatening condition.

The purpose of this study was to begin the development of this novel concept of US therapy. The use of clinically approved DUS systems for MCET likely will be sub-optimal without modification of the normal scanning modes for local targeting and for enhanced effectiveness of pulse regimes. For example, 10 min of myocardial contrast echocardiography in rats at 2 MPa peak rarefactional pressure amplitude (PRPA) with scans triggered every 4 beats during Optison infusion, visual scoring of histologic sections taken at the center of the scan plane indicated that only 10.8% of the area was involved in microlesions (Miller et al. 2005b). In addition, the rate of production of microbubble-associated bioeffects appears to decrease with time (Miller et al. 2010). This phenomenon may preclude the simple prolongation of treatment by clinical diagnostic systems to achieve therapeutic efficacy, especially in humans. The required cumulative microlesion level likely will be ~25% or more, which might be sufficient to reduce a hypertrophic septal wall of 20 mm, to a normal thickness of 15 mm in humans (Marian 2009). In this study, the influences of increasing pulse amplitude, pulse duration and intermittent trigger interval on microlesion effects were examined to identify promising modifications of DUS parameters to support therapeutic application.

MATERIALS AND 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. Male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 348 ± 41 g were anesthetized by intraperitoneal injection of a mixture of ketamine (90 mg kg^{-1}) and xylazine (9 mL kg^{-1}). A total of 45 rats were used for this study with nine rats lost from the study because of technical problems with the aiming and scoring process, and one due to anesthetic death. The left thorax was shaved and depilated for US transmission. A 24-gauge cannula was inserted into a tail vein for intravenous injections of contrast agent. Evans blue dye in saline (20 mg/mL) was injected intravenously at a dose of 100 mg/kg as a vital stain for cardiomyocytes (Miller et al. 2005a). The rats were mounted on a positioning board and electrocardiogram (ECG) needle electrodes were placed in the forelegs and left hind leg. The rat and board were then mounted for US exposures in a 37°C degassed and deionized water bath and aligned for exposure using an adjustable gantry.

The ECG signal was amplified (Model ECGA amplifier, Hugo Sachs Elektronik, Harvard Apparatus, March, Germany) and displayed on an oscilloscope (Model TDS 520 B, Tektronix Inc., Beaverton, OR, USA). The ECG was digitized (Powerlab 4/30, ADInstruments Inc. Colorado Springs, CO, USA) and analyzed with the aid of software (Chart Pro 5, v. 5.5.5, ADInstruments Inc.), which provided data on heart rate and the response to the ultrasonic therapy. The software partially automated the assessment of premature complexes (PCs), which coincided with the trigger of a therapeutic pulse sequence, followed by a compensatory pause.