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● *Original Contribution*

ULTRASONIC CAVITATION-ENABLED TREATMENT FOR THERAPY OF HYPERTROPHIC CARDIOMYOPATHY: PROOF OF PRINCIPLE

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Abstract—Ultrasound myocardial cavitation-enabled treatment was applied to the SS-16^{BN} rat model of hypertrophic cardiomyopathy for proof of the principle underlying myocardial reduction therapy. A focused ultrasound transducer was targeted using 10-MHz imaging (10 S, GE Vivid 7) to the left ventricular wall of anesthetized rats in a warmed water bath. Pulse bursts of 4-MPa peak rarefactional pressure amplitude were intermittently triggered 1:8 heartbeats during a 10-min infusion of a microbubble suspension. Methylprednisolone was given to reduce initial inflammation, and Losartan was given to reduce fibrosis in the healing tissue. At 28 d post therapy, myocardial cavitation-enabled treatment significantly reduced the targeted wall thickness by 16.2% ($p < 0.01$) relative to shams, with myocardial strain rate and endocardial displacement reduced by 34% and 29%, respectively, which are sufficient for therapeutic treatment. Premature electrocardiogram complexes and plasma troponin measurements were found to identify optimal and suboptimal treatment cohorts and would aid in achieving the desired impact. With clinical translation, myocardial cavitation-enabled treatment should fill the need for a new non-invasive hypertrophic cardiomyopathy therapy option. (E-mail: douglm@umich.edu)

Key Words: **Hypertrophic cardiomyopathy, Myocardial contrast echocardiography, Ultrasonic cavitation, Cardiac myocyte necrosis, Cardiac fibrosis.**

INTRODUCTION

The need for myocardial reduction arises for septal obstructive hypertrophic cardiomyopathy (HCM) and other ventricular hypertrophies, such as apical hypertrophy. HCM is one of the most common genetic cardiovascular diseases, occurring in more than 1 in 500 people (Maron and Salberg 2014; Semsarian et al. 2015) and places patients at risk for sudden death (Maron and Maron 2013). There is a wide range of variation in clinical presentation, with treatment tailored to each patient (Enriquez and Goldman 2014). Hypertrophy leads to obstruction of the left ventricular outflow pathway, either at rest or with provocation, in up to 75% of patients. About one-third of patients with obstruction remain symptomatic after pharmacologic therapy and are candidates for myocardial reduction (Marian 2009).

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Hypertrophy can occur in several regions of the myocardium, and is particularly troubling when restrictions occur in the left ventricular outflow tract (LVOT). Apical hypertrophy is also troubling and currently has no available corrective therapy (Jan et al. 2016). Surgical septal myectomy is presently the primary method for reduction of asymmetric septal hypertrophy with symptomatic LVOT (Enriquez and Goldman 2014; Gersh et al. 2011). Alternatives to surgery have been sought using various technologies. Thermal ablation with high-intensity focused ultrasound (Otsuka et al. 2007) and radiofrequency ablation (Lawrenz et al. 2011) have yet to provide a safe alternative. Transcatheter septal ablation with alcohol represents a viable alternative to surgery (Gersh et al. 2011; Leonardi et al. 2010). However, this procedure is associated with a relatively high incidence (~20%) of heart block requiring a permanent pacemaker (Marian 2009; Singh et al. 2016) and a substantial risk of serious arrhythmia (Nishimura and Ommen 2010). The immediate therapeutic effect of alcohol ablation may actually reflect akinesis of the region affected by alcohol ablation, with septal

thinning of about 24% developing 6 mo after treatment (Baggish et al. 2006; Dąbrowski et al. 2012). None of these techniques is fully satisfactory, suggesting that an innovative, safe and effective new therapy for this condition is warranted.

We have developed a novel ultrasonic technique called myocardial cavitation-enabled treatment (MCET), which employs intermittent pulsed ultrasound exposure with peak rarefactional pressure amplitudes about twice the guideline upper limit for diagnostic ultrasound (Miller et al. 2005) of the myocardium together with ultrasound contrast-agent microbubbles to produce cavitation nucleation and tissue injury. In our research thus far, a single-element focused transducer with fixed beam has been used, but larger volumes could be treated by beam scanning with a scanning system capable of high pulse amplitudes. This treatment produces scattered nucleation sites of ultrasound-induced injury within the focal zone primarily of cardiac capillaries, with leakage and small hemorrhage, and injury of the adjacent cardiomyocytes for supra-threshold ultrasound pressure amplitudes, leading to microscale surgical impacts of local cardiomyocyte necrosis and microscopic lesions. The microlesions accumulate with each intermittent exposure to achieve a desired total impact. In initial animal studies, MCET lesion creation was dose dependent and adjustable by manipulation of the ultrasound focus. When triggered at the end of systole, electrocardiogram (ECG) premature complexes (PCs) indicate microlesion occurrence (although arrhythmia could be avoided by triggering at the R wave without loss of efficacy), and the degree of injury is correlated with plasma troponin levels (Miller et al. 2014a, 2014b, 2015). Of note, cardiac function was preserved during therapy, and no conduction abnormalities, such as ST segment elevation, persisted beyond a few hours (Miller et al. 2014a; Zhu et al. 2015a). Additional studies in normal rats evaluated the acute effect and long-term maturation of the myocardium treated with MCET to assess the potential for therapeutic effect (Lu et al. 2016). These studies revealed significant increases in heart wall thickness a day after therapy, likely secondary to the inflammatory response to the injury. Wall thickness returned to normal 4 weeks after exposure, and pathology revealed significant fibrosis and depletion of myocytes in the treatment zone. These results suggested that MCET might be a feasible and relatively safe technique to reduce viable myocytes in thickened areas in patients with HCM, although no significant wall reduction was observed. Further studies are necessary to optimize long-term tissue reduction and to limit the initial inflammatory response.

Building on the previous work, this study was designed as a proof-of-principle evaluation of MCET in a rat model of HCM. The consomic Dahl SS rat with chromosome 16 from brown Norway rats (SS-16^{BN}) undergoes progressive left ventricular (LV) hypertrophy without hypertension (Chao et al. 1998; Klotz et al. 2006; Kriegel and Greene 2008). In this study, SS-16^{BN} rats underwent MCET to assess acute tolerance of the procedure. In addition, based on work focusing on reduction of fibrosis in myocardial infarction (de Carvalho Frimm et al. 1997; Roberts et al. 1985), steroids and angiotensin II receptor blocker were utilized to decrease the acute inflammation response and decrease the fibrosis seen after MCET therapy (Lu et al. 2016). Finally, a chronic test of MCET versus sham treatment was conducted utilizing steroid and anti-fibrotic adjuvants, with treatment evaluated by counting PCs and plasma troponin measurement. This study with a 28-d follow-up was conducted to assess the true impact on myocardial function and wall thickness in the treatment zone and provide the proof of principle for myocardial reduction therapy.

METHODS

Animal preparation

All *in vivo* animal procedures were conducted with the approval and guidance of the Institutional Animal Care and Use Committee of the University of Michigan, which conform to National Institutes of Health guidelines (*Guide for the Care and Use of Laboratory Animals*). In preliminary studies, Dahl SS rats (Charles River, Wilmington, MA, USA) were used owing to the widespread use of this strain as a model of human disease, including cardiomyopathy. Dahl SS rats were treated at 8–9 w of age with up to 4 w of follow-up. For the main study, SS-16^{BN} rats (Medical College of Wisconsin, Milwaukee, WI, USA) were used as a specific model of HCM without hypertension (Kriegel and Greene 2008). The veracity of this model for the human condition is only general, but there are few alternatives for a rat model of HCM (Shephard and Semsarian 2009). Although hypertrophy was evident in the SS-16^{BN} model, there was no ultrasonically identifiable outflow tract obstruction, nor pathologically identifiable areas of myofiber disarray, which are common features of the human disease. These rats were obtained soon after weaning and housed for up to 13 mo before treatment to allow development of the myocardial hypertrophy.

For ultrasound treatment, the rats were anesthetized by intraperitoneal injection of a mixture of ketamine (90 mg/kg) and xylazine (9 mL/kg), monitored by observation and pedal reflex. The left thorax was shaved and depilated for ultrasound transmission to the heart. A 24G 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 needle electrodes were placed in the forelegs and left hindleg for