Published in final edited form as:

Ultrasound Med Biol. 2016 July ; 42(7): 1541–1550. doi:10.1016/j.ultrasmedbio.2016.02.006.

Maturation of Lesions Induced by Myocardial Cavitation-Enabled Therapy

Xiaofang Lu, Douglas L. Miller, Chunyan Dou, Yiyang I. Zhu, Mario L. Fabiilli, Gabe E. Owens, and Oliver D. Kripfgans

University of Michigan Health System, Ann Arbor MI USA

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 by 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. Higher exposure used in group B yielded more substantial injury than lower exposure in group A. Treated rats in both group A and B had significant increases in wall thickness measured by echocardiography the next day, which returned to normal by the end of 6 weeks. Six weeks after ultrasound exposure, heart tissue samples showed tissue fibrosis in Masson's trichrome stained histology. Maturation of lesions involved fibrosis replacement, preserving structural tissue integrity. This study showed 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.

Keywords

Ultrasound therapy; hypertrophic cardiomyopathy treatment; ultrasonic cavitation microlesions; tissue reduction therapy

Introduction

Ultrasound contrast agents consist of suspensions of stabilized microbubbles which 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, cavitation response of microbubbles to ultrasound pulses can induce bio-effects in

Corresponding author: Xiaofang Lu, 3218 Medical Sciences Building I, University of Michigan Health System, 1301 Catherine Street, Ann Arbor MI 48109-5667, Tel: (734) 763-8855, FAX: (734) 764-8541, xiaofalu@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

mammalian tissue, which can be directed noninvasively for therapeutic purposes. Cavitation injury can be manipulated using different ultrasound methods for targeted cardiovascular therapy (Laing et al. 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 shown 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 US 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 as 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.

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 safe 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 the variation of timing of pulses relative to the cardiac cycle (Miller et al. 2014b), and by variation of ultrasound exposure parameters to allow advantageous combination of imaging and treatment targeting (Miller et al. 2015). The targeted accumulation and distribution of myocardial necrosis has been assessed using Evan's blue staining of injured cardiomyocytes in tissue histology slides for samples taken one day 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 of 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 weeks after MCET under two different ultrasound exposure parameter settings. The changes in the left ventricular morphology were followed by diagnostic imaging, and changes in the ECG were observed. The fraction of myocardial tissue destroyed and the resulting scar formation were evaluated.

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 of the University of Michigan. Three rats

were lost from the study due to technique problem with parameter setting in one rat and anesthetic deaths of 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 utilized 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 deionized 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 3314A function generator, Hewlett Packard Co., Palo Alto CA, USA), an arbitrary waveform generator for amplitude modulation of the pulse train (model 33220A, 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 (FOV) 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 FOV 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 (PRPA) of the pulses were measured using a calibrated hydrophone (model 805, Sonora Medical Systems Inc., 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 peak rarefactional pressure amplitude 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 envelope signal was either a 4 ms square or a 15 ms Gaussian function (Fig. 2). The pulse groups are shown in Table 1. The rat ECG signal was

amplified (Model ECGA amplifier, Hugo Sachs Elektronik, Harvard Apparatus, March, Germany) and displayed on an oscilloscope (Model TDS 520 B, Tektronic Inc., Beaverton, OR, USA). This setup allowed the modulated pulse bursts to be triggered from the ECG signal at approximately end of systole each 4 or 8 heart cycles, as described previously (Miller et al. 2014a).

Ultrasound contrast agent

The here employed ultrasound contrast agent was a laboratory replacement for Definity® (Lantheus Medical Imaging, Inc., N. Billerica MA, USA). It was created using a formula set as close as possible to that of the Definity. A lipid blend was made using powder forms of dipalmitoyl-sn-glycero-3-phosphate (DPPA, Sigma-Aldrich, Milwaukee WI, USA), dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, Avanti Polar Lipids, Alabaster, AL, USA), and dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-5000] (DPPE-PEG5000, Avanti Polar Lipids). The lipid were dissolved in a diluent consisting of 80% (v/v) phosphate buffered saline, 10% (v/v) propylene glycol, and 10% (v/v) glycerol at concentrations of 0.045 mg/mL, 0.4 mg/mL, and 0.3 mg/mL for DPPA, DPPC, and DPPE-PEG500, respectively. The mixture of lipids and diluent was heated to 70°C (i.e. above the transition temperature of DPPA) to solubilize the lipids. The resulting lipid blend was sterile filtered using a 0.22 µm filter and then aliquoted into used Definity vials that were cleaned/autoclaved. The headspace for each vial was filled with octafluoropropane (HC-218, PurityPlus, Metro Welding Supply, Detroit MI, USA) and the vial was then subsequently capped and sealed. The vials were shaken exactly as performed for Definity to produce the suspensions of stabilized microbubbles. The suspensions of microbubbles were then diluted in sterile saline in a 3 mL syringe, and infused via tail vein with a syringe pump at a rate of 5µL/kg/min, which was employed previously (Miller et al. 2014a) and approximates the maximal recommended dose for diagnostic application.

Experimental plan

The study was conducted with 2 groups of exposed rats (group A with lower exposure and group B with higher exposure, Table 1) and a sham group. Each group included 2 rats for acute study to validate the exposure in the Dahl/SS rats and 6 rats for long-term observation. Only long-term rats were included in the experimental analysis of the lesion progression.

For exposure, B-mode imaging was used to target the heart, and the exposure transducer was moved into place. The ECG signal was acquired, digitized, and analyzed with the aid of software (Chart Pro 5, v. 5.5.5, AD Instruments Inc., Colorado Springs, CO, USA), as described previously (Miller et al. 2014a). After aiming, the ECG was recorded for 1 min pre-exposure, 5 or 10 min during exposure with infusion of diluted contrast agent, and 1 min post-infusion with continued exposure. After treatment, the electrodes and the catheter were removed, and the rat was dried and monitored in a warmed cage until fully awake. The ECG analysis provided data on heart rate and the arrhythmia response to the ultrasonic therapy. The software partially automated the assessment of premature ventricular complexes (PVCs), which coincided with the trigger of the therapeutic pulse sequence, followed by a compensatory pause, which can usually be seen after the PVC. The rat sham group was

exposed using either set of parameters for 5 or 10 min without infusion of contrast agent, followed by 5 or 10 min infusion of contrast agent with no ultrasound exposure.

Blood samples were collected from the tail vein before exposure and 4 hours after exposure and centrifuged. Plasma was taken for use in a troponin I assay, which is an indicator of lethal cardiomyocyte injury.

To compare microlesion production in the Dahl/SS rats to the Sprague-Dawley rats in acute procedures, Evans blue dye (E1129, Sigma-Aldrich Co, St. Louis MO, USA) in saline (20 mg/ml) was injected IV via tail vein at a dose of 100 mg/kg as a vital stain for cardiomyocytes. Lethal injury of cardiomyocytes was scored on the basis of Evans blue staining, as described previously (Miller et al. 2005; Miller et al. 2014a). Briefly, the hearts were removed the day after exposure and cleared of most of the blood with heparin-saline. The hearts were trimmed to remove the atria, and frozen in freezing compound (Tissue-Tek, Sakura Finetek USA Inc. Torrance CA, USA) on dry ice and stored at -80°C . The hearts were sectioned to provide up to forty 10 μm thick sections at 200 μm intervals. Each slide was scored blindly using fluorescence microscopy and a final stained cardiomyocyte score (SCS) was calculated as the sum of the scores for the individual slides. For these therapeutic exposures, there were too many close packed stained cells to count individually; hence, the SCS was a qualitative measure of overall injury.

For long-term observation, left ventricular wall thickness and ejection fraction were measured before exposure, the next day and once a week for a total period of 6 weeks using a linear array probe (GE Vivid 7 with i13L probe, GE Vingmed Ultrasound, Horten, Norway). At the end of 6 weeks, the hearts were removed. After cannulation of the aortas, the hearts were connected to a column of neutral buffered formalin set to a height equivalent to a pressure of 15 mmHg and immersed in formalin. This way, the hearts were fixed approximately in end-diastolic configuration (Whittaker et al. 2000). The hearts were cut parallel to the atrioventricular groove into 4–5 slices and photographed. For one slice that included the treatment area, histology was performed for H&E staining and Masson's trichrome staining for fibrosis. The distribution and accumulation of the myocardial fibrosis formation were assessed by a computer-aided quantitative method.

Statistics

Results are reported as the means plus/minus one standard deviation, or plotted with standard error bars. One-way ANOVA tests were used to compare wall thickness measurements between 3 groups at the end of 6 weeks. Student's t-tests were used to compare means of the PVCs percentages, 4-hour troponin levels and microlesion fractions between group A and group B, as well as measurements of wall thickness between baseline and one day after exposure for each exposed group. Statistical significance is assumed at $P < 0.05$. Correlation analysis was used to quantify the association between 4-hour troponin level and fraction of fibrotic tissue within the treatment area in both exposed groups (QI Macros for Excel 2013, KnowWare International Inc., Denver, CO).

Results

Acute tests

A red and blue circular area was seen at gross observation on the anterior surface of each heart that was removed the day after exposure, indicating the ultrasound-induced damage. The areas were much larger in group B (pulse duration of 10 pulses, 4 ms square modulation, 1:8 trigger for 10 min) than those in group A (pulse duration of 5 pulses, 15 ms Gaussian modulation, 1:4 trigger for 5 min), see Fig 3. There was no obvious injury on the hearts of sham rats.

SCSs in the samples from group B (34323 and 37570 in 2 rats with higher exposure) were much higher than those in the samples from group A (6899 and 12116 in 2 rats with lower exposure). No EB-stained cells were found in sham rats. These acute effects in the Dahl/SS rats were commensurate with the previous results from Sprague-Dawley rats.

Long term observation of maturation

Results for percentages of PVCs over trigger times during exposure for each group are shown in Fig. 2. Group B had significantly higher percentages of PVCs than group A ($P=0.003$), while no PVC was found in the sham group. Results for 4-hour troponin I levels for each group are shown in Fig. 4. Group B had significantly higher troponin I levels than group A 4 hours after the exposure ($P<0.001$), indicating more myocardial damage in group B (Fig. 5). Trace troponin levels near zero were detected in sham rats.

Although variable, there were typical ECG changes related to myocardial injury in both group A and B, which evolved with time. These ECG changes included ST segment elevation right after the exposure, followed by ST segment suppression and T wave inversion which persisted for various periods of time. Examples of these ECG waveforms from each group were shown in Fig. 6.

Left ventricular wall thickness changes measured in echocardiography follow-up over 6 weeks for each group were shown in Fig 7. Rats in both group A and B had a significant increase in wall thickness on the next day after exposure (from 1.72 ± 0.06 mm to 2.37 ± 0.36 mm in group A, $P=0.007$; from 1.68 ± 0.05 mm to 2.75 ± 0.18 mm in group B, $P<0.001$). Increased wall thickness gradually returned to pre-therapy levels by the end of 6 weeks. There was no difference in wall thickness between sham, group A and group B after 6 weeks when the rats were euthanized for histological evaluation ($P=0.166$).

At the end of the study, six weeks after ultrasound exposure, heart tissue samples were taken for histological evaluation. Evident scar development could be seen within the target region of the hearts from group B, see Fig. 8. The tissue fibrosis was evident in Masson's trichrome stained slides. Blue stained areas (collagen) were indicative of fibrosis formation in the lesion areas relative to the red background tissue, see Fig. 8. For each Masson's stained slide, the most dense lesion region was used to calculate the fractional lesion, i.e. blue area over 3 mm wide window constrained ventricular wall area. The window width was approximated from average *in vivo* macrolesion width (Zhu et al, 2015b). As shown in Fig. 9, the transparent region is where the fibrotic tissue fraction was calculated. This fraction

within the treatment volume indicates the fraction of cardiomyocyte removal, which is much higher in group B compared to group A (0.34 ± 0.11 versus 0.09 ± 0.06 , $p < 0.001$). A moderate positive correlation was found between 4-hour troponin levels and the fraction of fibrotic tissue (correlation coefficient is 0.669), shown in Fig. 10. Possible narrowing of the LV wall was noted in a few slides from exposed rats in group B, see Fig. 8. However, this effect was not consistent and was not quantified due to the difficulty in assessing LV wall variations in these irregular tissue sections. The fibrotic regions appeared to be typical of myocardial repair following injury, with myofibroblasts present in the area devoid of cardiomyocytes, as shown in Fig. 11.

Discussion

In this study, in order to evaluate the long term outcome and safety of MCET, two different sets of enhanced therapeutic parameters were used for MCET to induce injury on rat hearts. PRPA of 4 MPa was used because our previous study showed PRPA of 2 MPa had no evident changes in the ECGs indicative of cardiac injury. Increased trigger intervals (from 1:4 to 1:8) and pulse durations (from 5 to 10 cycles) seemed to increase bio-effects (Miller et al. 2014a). Moreover, square envelope modulation at the highest peak rarefactional pressure amplitude has a larger effective volume in terms of integrated negative pressure over time, compared to Gaussian modulation (Zhu et al, 2015b). Based on these findings, we chose a set of parameters with higher effect (pulse duration of 10 cycles, 4 ms square modulation, 1:8 trigger for 10 min) and another with lower effect (pulse duration of 5 cycles, 15 ms Gaussian modulation, 1:4 trigger for 5 min). The difference between the two groups illustrates the range of therapeutic impact which can be achieved by respective exposure parameter selection. As expected, higher exposure used in group B yielded more substantial injury than lower exposure in group A. This was confirmed by PVC percentages during the exposure, troponin levels at 4 hours as well as the number of EB-stained myocardial cells scored the next day, and the extent of fibrosis after 6 weeks.

All rats survived the exposure and the 6-week period of follow-up, indicating that MCET is a fairly safe approach to be used for treatment purposes. In our previous study, lung injury was found in some of the sham-exposed rats possibly because of exposure from the therapy beam that had gone through the heart (Miller et al. 2014a). However, no obvious lung injury was found upon euthanasia, 6 weeks after exposure, in the present study. Lung injury might have faded away after 6 weeks, if there was any. The day after exposure, the increase in wall thickness in both exposed groups indicates an acute inflammatory reaction within the tissue. In our model, cell injury was scattered and the intrinsic vasculature remained intact. This might help to enhance the recruitment of inflammatory cells to the site and the progression of acute inflammation. This acute wall swelling may be a problem in the presence of the left ventricular outlet obstruction, since a swelling septum due to an acute inflammatory reaction within the exposed area might exacerbate the obstruction. However, the clinical treatment plan would likely involve a much smaller fraction of the entire myocardium than the present rat heart treatments.

Masson's staining showed up to 49% tissue in the center of the treated area was involved in fibrotic lesions in the high exposed group B. The lesions were scattered and randomly

distributed in the anterior wall without gross tissue structure destruction. This may be less severe for the tissue reduction in the treatment of HCM with fewer side effects, compared to traditional invasive septal reduction. Although the percentage of tissue damage is substantial for group B, no obvious shrinkage of wall thickness was observed when measured with echocardiography. No consistent difference in wall thickness between sham, group A and group B was found on histology, 6 weeks after treatment, although some indications of wall thinning were noted in the high exposure group. The unevenness and variation of the left ventricular wall thickness in the histological sections preclude the identification and measurement of small changes in wall thickness. Damaged microlesion areas seemed to be almost completely replaced by fibrotic tissue formation following the acute inflammation after cavitation-induced injury. This is characteristic of the normal replacement process in which fibrosis and scar preserve the structural integrity of the heart, for example after injury from ischemia.

This study showed that the myocardial injury noted previously after one day (Miller et al. 2015) progresses into permanent loss of myocardial tissue. The parameter set of 4 MPa PRPA amplitude, 10 cycles burst of ultrasound exposure with square modulation, 1:8 ECG trigger produced permanent injury sufficient for possible tissue reduction therapy. Six weeks after the treatment, myocardial lesions were mostly replaced by patches of fibrous tissue. No actual reduction of heart wall thickness was measurable in these healthy rats, which could be undesirable for HCM treatment. However, the inflammation and replacement scar volume seen here may be amenable to pharmaceutical intervention. Roberts et al. (1985) found that high dose methylprednisolone reduced scar thickness in experimental myocardial infarction in rats. This treatment also reduces the initial inflammatory response, which may be helpful in ameliorating the potential for exacerbation of LV outflow obstruction by the inflammatory swelling. Another potentially useful intervention may be blood pressure control during maturation. De Carvalho Frimm et al. (1997) showed that treatment with Losartan, an angiotensin II receptor blocker, daily for four weeks beginning one day after induction of infarct by coronary artery occlusion, significantly reduced fibrosis and scar thickness. For the MCET method, this treatment might reduce fibrosis volume and allow for improved tissue volume reduction.

Finally, a more appropriate comparison for MCET than surgical myectomy may be alcohol septal ablation (ASA). ASA is a minimally invasive procedure in which a catheter is threaded through the left anterior descending artery into the septal perforator, which is isolated by a balloon (El Masry and Breall, 2008). Following assessment of the area supplied by the artery, such as by contrast enhanced ultrasound, several milliliters of ethanol are injected to produce a localized infarct defined by the perfused tissue volume, which typically produces immediate symptomatic relief. This occurs without septal thinning, possibly owing to akinesis of the effected region, which may improve the dynamic motion and LVOT gradient. Septal thinning develops gradually 3–12 month after the procedure. Reduction of the interventricular septum from 23.7 to 18.0 mm has been reported 6 months after ASA (Dabrowski et al. 2012), and the treatment may result in reduced myocardial mass involving even remote regions (van Dockum et al. 2006). The most common serious complication is disruption of the conduction system, which may include permanent heart block requiring permanent pacemaker implantation. The ASA procedure results in a volume of necrosis,

including occlusion of the artery. This volume progresses to myocardial fibrosis and scar (Baggish et al. 2015), although the healing process is somewhat different from that arising from ischemia, in that minimal phagocytosis and removal of the necrotic cardiomyocytes occurs (Raute-Kreinsen, 2003). By comparison the MCET method produces a much lower impact on the conduction system and blood vessels, and a more defined region of treatment set by the placement of the ultrasonic focus. ECG analysis in this study revealed evidence of myocardial cell death with changes consistent with an old infarct by 6 weeks but no evidence of AV conduction loss or heart block. MCET also could potentially treat hypertrophied regions of the heart other than the septum. This reduced impact may reduce the significant adverse consequences, which are seen for some ASA patients (ten Cate et al. 2010). This study in relatively normal healthy heart tissue may not present a reasonable picture of the outcome in the hypertrophic myocardium, which may also provide rapid symptomatic relief without rapid wall thinning, followed by healing and gradual remodeling to advantageously reduce tissue volume. Research is needed on the use of MCET on animal models of HCM, such as the SS-16BN/Mcwi strain (Kriegel and Greene, 2008), a rat model of left ventricular hypertrophy, to assess the effect on abnormal myocardial tissue and on modification of excessive LVOT gradients in larger animals.

Acknowledgments

This work was supported by PHS grant HL114595 awarded by the National Institutes of Health, DHHS.

References

- Baggish AL, Smith RN, Palacios I, Vlahakes GJ, Yoerger DM, Picard MH, Lowry PA, Jang IK, Fifer MA. Pathological effects of alcohol septal ablation for hypertrophic obstructive cardiomyopathy. *Heart*. 2006; 92:1773–1778. [PubMed: 16807273]
- ten Cate FJ, Soliman OI, Michels M, Theuns DA, de Jong PL, Geleijnse ML, Serruys PW. Long-term outcome of alcohol septal ablation in patients with obstructive hypertrophic cardiomyopathy: a word of caution. *Circ Heart Fail*. 2010; 3:362–369. [PubMed: 20332420]
- De Carvalho Frimm C, Sun Y, Weber KT. Angiotensin II receptor blockade and myocardial fibrosis of the infarcted rat heart. *J Lab Clin Med*. 1997; 129:439–46. [PubMed: 9104887]
- D browski M, Chojnowska L, Małek L, Spiewak M, Ku mierzcyk B, Koziarek J, Klisiewicz A, Mi ko J, Witkowski A. An assessment of regression of left ventricular hypertrophy following alcohol ablation of the interventricular septum in patients with hypertrophic cardiomyopathy with left ventricular outflow tract obstruction. *Kardiol Pol*. 2012; 70:782–788. [PubMed: 22933209]
- van Dockum WG, Kuijer JP, Götte MJ, Ten Cate FJ, Ten Berg JM, Beek AM, Twisk JW, Marcus JT, Visser CA, van Rossum AC. Septal ablation in hypertrophic obstructive cardiomyopathy improves systolic myocardial function in the lateral (free) wall: a follow-up study using CMR tissue tagging and 3D strain analysis. *Eur Heart J*. 2006; 27:2833–2839. [PubMed: 17098761]
- Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, Naidu SS, Nishimura RA, Ommen SR, Rakowski H, Seidman CE, Towbin JA, Udelson JE, Yancy CW. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2011; 124:2761–2796. [PubMed: 22068435]
- Kriegel AJ, Greene AS. Substitution of Brown Norway chromosome 16 preserves cardiac function with aging in a salt-sensitive Dahl consomic rat. *Physiol Genomics*. 2008; 36:35–42. [PubMed: 18940898]
- Laing ST, McPherson DD. Cardiovascular therapeutic uses of targeted ultrasound contrast agents. *Cardiovasc Res*. 2009; 83:626–635. [PubMed: 19581314]

- Maron BJ, Maron MS, Semsarian C. Genetics of Hypertrophic Cardiomyopathy After 20 Years. *Journal of the American College of Cardiology*. 2012; 60:705–715. [PubMed: 22796258]
- El Masry H, Breall JA. Alcohol septal ablation for hypertrophic obstructive cardiomyopathy. *Curr Cardiol Rev*. 2008; 4:193–197. [PubMed: 19936195]
- Miller DL, Li P, Dou C, Gordon D, Edwards CA, Armstrong WF. Influence of contrast agent dose and ultrasound exposure on cardiomyocyte injury induced by myocardial contrast echocardiography in rats. *Radiology*. 2005; 237:137–143. [PubMed: 16183929]
- Miller DL, Dou C, Owens GE, Kripfgans OD. Optimization of ultrasound parameters of myocardial cavitation microlesions for therapeutic applications. *Ultrasound Med Biol*. 2014a; 40:1228–1236. [PubMed: 24613640]
- Miller DL, Dou C, Owens GE, Kripfgans OD. Timing of high-intensity pulses for myocardial cavitation-enabled therapy. *J Ther Ultrasound*. 2014b; 2:20. [PubMed: 25279221]
- Miller DL, Dou C, Lu X, Zhu YI, Fabiilli ML, Owens GE, Kripfgans OD. Use of Theranostic Strategies in Myocardial Cavitation-Enabled Therapy. *Ultrasound Med Biol*. 2015; 41:1865–1875. [PubMed: 25890888]
- Raute-Kreinsen U. Morphology of necrosis and repair after transcatheter ethanol ablation of septal hypertrophy. *Pathol Res Pract*. 2003; 199:121–127. [PubMed: 12812312]
- Roberts CS, Maclean D, Maroko P, Kloner RA. Relation of early mononuclear and polymorphonuclear cell infiltration to late scar thickness after experimentally induced myocardial infarction in the rat. *Basic Res Cardiol*. 1985; 80:202–209. [PubMed: 4004727]
- Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2015; 65:1249–1254. [PubMed: 25814232]
- Slikkerveer J, Veen G, Appelman Y, Royen N, Kamp O. Therapeutic application of ultrasound: contrast-enhanced thrombolysis in acute ST-elevation myocardial infarction; the Sonolysis study. *Neth Heart J*. 2011; 19:200–205. [PubMed: 21475732]
- Unger E, Porter T, Lindner J, Grayburn P. Cardiovascular drug delivery with ultrasound and microbubbles. *Adv Drug Deliv Rev*. 2014; 72C:110–126. [PubMed: 24524934]
- Whittaker P, Patterson MJ. Ventricular remodeling after acute myocardial infarction: effect of low-intensity laser irradiation. *Lasers Surg Med*. 2000; 27:29–38. [PubMed: 10918290]
- Zhu YI, Miller DL, Dou C, Kripfgans OD. Characterization of macrolesions induced by myocardial contrast enabled therapy (MCET). *IEEE Trans Biomed Eng*. 2015a; 62:717–727. [PubMed: 25347871]
- Zhu YI, Miller DL, Dou C, Lu X, Kripfgans OD. Quantitative Assessment of Damage During MCET: A Parametric Study In Vivo in a Rodent Model. *Journal of Therapeutic Ultrasound*. 2015b; 3:18. [PubMed: 26478815]

Figure 1.

Schematic illustration of the positioning setup of the imaging probe and the therapeutic transducer. For targeting, the rat heart was located at 2.25-cm depth at the edge of the sector, as shown on the left side. Subsequently, the therapeutic transducer was moved in so that the beam followed the same path (dotted grey line) as the previously identified image line (solid grey line), as shown on the right side. The heart was right in the focal zone of the therapeutic transducer (3.8-cm focal length), and shown at the center of the image sector at 2.75-cm depth (dashed grey line). The solid black arrow line on the left side showed the movement of the therapeutic transducer (1.9 cm).

Figure 2.
Sequence of the triggered pulse bursts for the square modulation (top) and the Gaussian modulation (bottom).

Figure 3.

Heart images (1× magnification) taken the day after therapy. The left image represents a sham case with no evident injury. The middle image represents a case with moderate injury (group A) and the right image a case with substantial injury (group B). Scale bar 1 mm.

Figure 4.

Premature complexes percentages for groups A, B and sham. Group B had significantly higher percentages of PVCs than group A ($P=0.003$). No PVC was found in the sham group. All groups represent a cohort with $n=6$.

Figure 5.

Results of the 4-hour plasma Troponin I for groups A, B and sham. Group B had significantly higher troponin I levels than group A, 4 hours after the exposure ($P < 0.001$). Trace troponin near zero was detected in sham rats. All groups represent a cohort with $n=6$.

Figure 6.

ECG records from pre-treatment, post-treatment, next day and 6 weeks after exposure. The upper row was from a case in group A, showing a slight elevation of ST segment post-treatment, followed by ST segment suppression the next day, which returned to normal by 6 weeks after exposure. The lower row was from a case in group B, showing a large elevation of ST segment post-treatment (thick arrow), followed by ST segment suppression and T wave inversion (thin arrow) the next day, which persisted 6 weeks after exposure.

Figure 7.

Left ventricular wall thickness changes from echocardiographic measurements for each group. Rats in both group A and B had a significant increase in wall thickness the next day after exposure, which gradually returned to normal by the end of 6 weeks. There was no difference in wall thickness between sham, group A and group B, 6 weeks after exposure.

All groups represent a cohort with n=6.

Figure 8.

Heart specimen photographs and microscopy images (including zoomed sections showing Masson's trichrome stained cells) from sham, group A and B (from left to right), respectively. The upper row were 1× magnification photographs taken 6 weeks after exposure, scale bar 1 mm; the middle row were 1× magnification bright field images of Masson's stained slides, arrow points to possible tissue shrinkage within damaged area, scale bar 1 mm; the lower row were 10× magnification bright field images of Masson's stained slides, scale bar 100 μ m.

Figure 9.

Quantification of the fibrotic tissue fraction within the treatment volume in Masson's stained slides. Transparent regions are the most dense lesion cloud ventricular wall regions where densities were calculated. Those regions are constrained by 3 mm wide windows. Slides from group A and B were shown on the left and right side, respectively. Scale bar 1 mm.

Figure 10.

A moderate positive correlation was found between the fibrotic tissue fraction and 4-hour troponin levels with a correlation coefficient of 0.669.

Figure 11.

Higher magnification view of a relatively large fibrotic region in the heart of a case from Group B with Masson's trichrome staining. Surviving cardiomyocytes are stained red, while collagen is stained blue. Within the blue fibrotic areas, the dark stained nuclei likely show the presence of myofibroblasts. Scale bar 20 μm .

Table 1

Exposure parameters for each group

Group ID	PRPA (MPa)	PD (cycles)	Modulation	Intervals (× RR)	Treatment duration (min)
A	4.0	5	15 ms Gaussian	4	5
B	4.0	10	4 ms square	8	10

PRPA = peak rarefactional pressure amplitude

PD = pulse duration

[×]RR = trigger interval in number of heartbeats