Published in final edited form as:

Ultrasound Med Biol. 2014 June ; 40(6): 1228–1236. doi:10.1016/j.ultrasmedbio.2014.01.001.

Optimization of Ultrasound Parameters of Myocardial Cavitation Microlesions for Therapeutic Application

Douglas L. Miller, Chunyan Dou, Gabe E. Owens, and Oliver D. Kripfgans

University of Michigan Health System, Ann Arbor MI USA

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 6 groups. ECG 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.

Keywords

Myocardial Contrast Echocardiography; Arrhythmia; Cardiac Myocyte Necrosis; Hypertrophic Cardiomyopathy

Introduction

Suspensions of stabilized microbubbles have been approved as commercial contrast agents for ultrasound 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 (Miller et al. 2008). These cavitation bioeffects have the potential to be

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Corresponding author: Douglas Miller 3240A Medical Sciences Building I University of Michigan Health System 1301 Catherine Street Ann Arbor MI 48109-5667 Tel: (734) 647-3344 FAX: (734) 764-8541 douglm@umich.edu.

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harnessed for therapeutic purposes. Potential cardiovascular applications presently under study include directed delivery of gases, drugs, genes and 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 scarring over about 6 weeks 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 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 pharmacological 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 ultrasound 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). Radio-frequency 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 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 ultrasound therapy. The use of clinically approved diagnostic ultrasound (DUS) systems for MCET likely will be suboptimal 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 histological 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 (IP) 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 9 rats lost from the study due to technical problems with the aiming and scoring process, and one due to anesthetic death. The left thorax was shaved and depilated for ultrasound transmission. A 24 gauge cannula was inserted into a tail vein for IV injections of contrast agent. Evans blue dye in saline (20 mg/ml) was injected IV 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 ECG needle electrodes placed in the forelegs and left hind leg. The rat and board were then mounted for ultrasound 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, March FRG) and displayed on an oscilloscope (Model TDS 520B, 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. Colorado Springs, CO USA), 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.

Ultrasound

Ultrasound exposure was provided by a laboratory system with guidance by diagnostic ultrasound imaging, similar to systems described previously (Miller et al. 2007, Miller et al. 2009). The laboratory exposure system consisted of a transducer (Panametrics A3464, Olympus, Waltham, MA, USA), a power amplifier (A-500, Electronic Navigation Industries, Rochester NY), a function generator for generating a pulse train (model 3314A function generator, Hewlett Packard Co., Palo Alto CA) and an arbitrary waveform generator (model 33220A, Agilent Technologies, Loveland CO) for modulation of the pulse train to produce pulse-bursts. The damped 1.9 cm diameter transducer had a 3.8 cm focus, with a -6 dB beam diameter of 3.5 mm. The focused beam was aimed with the aid of images on a diagnostic ultrasound machine (GE Vingmed System V with FPA10 probe, General Electric Corp., Cincinnati OH USA) operated at 8 MHz with 5 cm focal depth. The imaging probe and therapeutic transducer were clamped together at a 37 degree angle so that the position of the transducer focus was located in the image. For targeting, the rat heart was located at 2.25 cm depth at the edge of the sector scan, and then repositioned at the center of the image at 2.75 cm depth for exposure, see Fig. 1. This maneuver assured that the transducer was aimed between ribs, and through the window between the sternum and lungs. The vertical position was adjusted so that the beam impinged approximately at the middle of the left ventricle.

Two pulse generators and an oscilloscope were used to produce a burst of 8 pulses with a 4 kHz pulse repetition frequency and to trigger the burst from the ECG signal at approximately end of systole, with a specific interval measured in heart beats. One generator produced a continuous pulse train, while the other generator produced an amplitude modulation signal to create the short burst of pulses. The trigger-signal position was set via the hold-off and delay functions of the oscilloscope. Seven groups of rats were treated under the different pulsing conditions listed in Table 1. The value of PRPA divided by the square root of the 1.5 MHz frequency, which is indicative of an equivalent Mechanical Index (MI), was 1.6 for 2 MPa and 3.3 for 4 MPa. For reference, the guideline upper limit for diagnostic ultrasound is $MI = 1.9$ (FDA, 2008). The PRPAs of the pulses were measured and set using a calibrated hydrophone (model 805, Sonora Medical Systems Inc., Longmont CO USA). The recorded pulse waveform data was used for calculating the spatial-peak pulse-average intensity by the standard method (AIUM/NEMA, 2007). For the 4 MPa 10 cycle pulses, the measurement was uncertain due to possible cavitation activity in the measurement water bath. The spatial-peak pulse-average intensities were well above the guideline (Track 1) limit of 190 W/m^2 for diagnostic ultrasound (FDA, 2008). However, the intermittent pulse bursts resulted in a low spatial-peak temporal-average intensity, well below the FDA guideline of 720 mW/cm^2 , which would produce negligible heating. For example, the 892 W/cm^2 pulse average intensity would be greatly reduced by the pulse duty cycle (2.48 %) and by the ratio of burst duration (2 ms) to repetition interval (about 1 s for 4 RR intervals), to only about 44 mW/cm^2 temporal average intensity.

For exposure, the image was used to target the heart, and then the exposure transducer was moved into place. Definity® (Lantheus Medical Imaging, Inc., N. Billerica, MA) ultrasound contrast agent was diluted 100:1 in sterile saline in a 3 ml syringe, and infused via tail vein

at 0.5 ml/kg/min of diluted agent. This gave 5 μ l/kg/min of agent infusion, which would be at the high end of recommended infusion rates for human imaging. After targeting, the ECG was recorded for 1 min pre-exposure, 5 min during exposure with infusion of diluted contrast agent, and 1 min post-infusion with continued exposure. In addition, the ECG was recorded for 1 min before heart removal the next day. The appearance of contrast agent in the heart, and its perturbation by a therapy pulse burst are shown in Fig. 1. The imaging system was operated with a -12 dB power level (On screen MI=0.3) to minimize perturbation of the microbubbles when used briefly during exposure (during aiming, no microbubbles were present).

Cardiomyocyte scoring

Lethal injury of cardiomyocytes was scored on the basis of Evans blue staining, as described previously (Miller et al. 2005a). The hearts were removed the day after exposure and cleared of most 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. These were stored in a -80 freezer until sectioning and scoring. Each heart was sectioned to provide samples covering the entire exposed myocardial volume. Twenty-five 10 μ m thick sections were cut each 200 μ m into the sample to cover about 5 mm from the exposed central portion of the heart. Each slide was scored blind and a final result obtained by summing the slide scores to obtain the stained cardiomyocyte score (SCS). For a low density of scattered stained cardiomyocytes, the score represented an approximate cell count; however, for the therapeutic exposures, there were too many close packed stained cells to count. Therefore, the cell score in this study was a relative measure of overall injury. During scoring, a problem was discovered, in which the affected area of tissue sometimes fell outside the sampled region, resulting in the exclusion of 9 rats from the study as noted above. Subsequently, the transducer aim was improved, the trimming of the sample was used to more closely crop to the exposed region and up to 40 sections were made of each heart.

Experimental Plan

The study was conducted in 6 exposed groups of 5 rats each and a sham group of 4 rats. PRPAs were 2 or 4 MPa, pulse durations were 5 or 10 cycles, and trigger intervals were 1:4 or 1:8 heartbeats, as listed in Table 1. The base condition of 2 MPa, 5 cycle pulses and 1:4 trigger interval approximated parameters for diagnostic myocardial contrast echocardiography. The increased PRPA, pulse duration and pulse interval, which allows more complete refill of the tissue with microbubble-containing blood, were each expected to enhance the cardiomyocyte effects toward the goal of therapeutic efficacy. For the 1:4 interval, exposures were 5 min, while for the 1:8 interval, the exposure duration was extended to 10 min, so that approximately the same number of pulse-bursts were delivered for all exposures. Data were collected for each rat on PCs as a fraction of triggers in the ECG recording. Each rat heart was photographed and the stained cell scores collected to assess injury. The PCs are indicative of the occurrence of lethal cardiomyocyte injury (Miller et al. 2011), and thus the PCs were hypothesized to give a real-time indication of the therapeutic effect. Results are reported as the means plus or minus one standard deviation, or plotted with standard error bars. Statistical analysis was performed (SigmaPlot for Windows

V. 11.0, Systat Software Inc., San Jose CA, USA). Student's t-tests were used to compare means of the measured parameters, with statistical significance assumed at $P < 0.05$. In addition, ANOVA was used to examine the dependence of results on the different parameters of pulse amplitude, pulse duration and pulse-burst trigger interval.

Results

The heart rate was not significantly changed for any group, and overall was 281 ± 27 bpm for pre-exposure and 288 ± 36 bpm for post-exposure monitoring. The ECG waveform was used to evaluate indication of cardiac injury, but aside from PCs (described below) no consistent electrocardiographic changes were observed between pre-, post- and 1-day monitoring periods in any treatment groups. However, a few animals appeared to have important changes in the ECG waveform with regard to heart injury. Examples of these ECG waveforms are shown in Fig. 2, together with photographs of the hearts. A sham heart (group A) had no changes suggestive of myocardial injury. A heart with moderate injury induced by MCET (group E) shows a clear S-T segment elevation evident post exposure that is indicative of ischemia or injury, but resolves after 24 hr. A heart showing substantial microlesion injury (group G) shows substantial elevation of the S-T segment immediately post exposure and has T-wave inversion after 24hrs that is suggestive of substantial and likely permanent injury. Of note, no groups with 2 MPa PRPA had evident changes in the ECGs.

Results for PCs are listed in Table 1 for each group. All of the exposures had PCs significantly greater than the null results for the shams. A two-way analysis of variance (ANOVA) for the 4 exposures with 5 cycle pulses showed that the differences due to the different PRPA values was statistically significant ($P = 0.013$). However, the different intervals did not produce a significant influence on results, nor was the strong influence of PRPA affected by the interval. A two-way analysis of variance (ANOVA) for the 4 exposures with burst-intervals of 1:8 heartbeats showed that the differences due to the varying PRPA values again was statistically significant ($P = 0.001$). The different pulse durations seemed important but this did not achieve statistical significance ($P = 0.075$). In addition, the influence of PRPA was not significantly affected by the pulse duration ($P = 0.065$).

An important factor during exposure is the fraction of triggered exposure bursts, which elicit PCs, and the variation of this fraction with time. A decrease in this fraction during the exposure period would be indicative of decreasing effectiveness with time, a "saturation" phenomenon, which could greatly impair the treatment effectiveness. A breakdown of the PCs is shown in Fig. 3 to compare the fraction of exposure bursts with PCs in the minute of exposure beginning 15 s after the start of infusion to the last minute of exposure with infusion. Generally, there was a significant reduction in PCs for the three 2 MPa exposure conditions ($P < 0.02$), but not for the 4 MPa exposure conditions ($P > 0.05$). Three-way ANOVAs of the groups with 5 cycle pulse durations and the groups with 1:8 intervals showed that both the start-end time selection and the PRPA significantly influenced the results, and that the start-end difference was significantly influenced by the PRPA. The use

of relatively high therapeutic pressures therefore appears to mitigate saturation and allow enhanced efficacy.

The SCS results are listed in Table 1. An important feature of the results is the large variation within groups. Fractional standard deviations exceed 50%, and even 100% for group B. The SCSs were much more variable than the ECG results for PCs. The initial problems, noted above, could not explain this variability because the affected individual rats were excluded from the study, and the method improved. Some of the variation may have been due to small differences in the targeting of the beam. Fig. 4a shows a distinct beam path marked by stained cells in a heart from group F, which passed along the septum, through the heart. For other hearts, the anterior wall of the left ventricle was strongly affected, see Fig. 4b from group B, while the posterior wall was not, which probably indicates strong attenuation by the microbubble-filled blood in the ventricle with an acoustic shadow on the posterior side. Very strong impacts were also noted in some hearts, as shown in Fig. 4c. This region appears to have all the cardiomyocytes stained, which resembled an infarct, except that the stained region corresponded to the beam area, rather than to the region perfused by a specific coronary artery. Two-way ANOVA for the groups with 5 cycle pulses showed that the PRPA had a significant influence on results ($P < 0.02$), but the trigger interval did not. The PRPA also was most important ($P < 0.05$) for the groups with 1:8 interval, with no significant influence for the pulse duration.

The results for SCS as a function of the number of PCs are plotted in Fig. 5, to assess the predictive ability of the PCs for the microlesion bioeffect. The group letter is displayed inside each symbol with bi-directional error bars. The total number of PCs serves as a dose parameter in Fig. 5, for the bioeffect, although it should be noted that the maximum number of PCs was about 350 (i. e. 100 percent of triggered bursts) for this test. Unfortunately, there was a large variation in the SCSs for a specific PC count, which indicates that the PC occurrence is a poor dose parameter for the actual numbers of dead cells. The solid line in Fig. 5 is a linear regression on the individual data points, with 95% confidence intervals shown as dotted lines. For this linear regression, the correlation coefficient was $r^2 = 0.34$. The infarct-like impacts on the heart, which had very large scores, were seen for group G, which has the largest standard error bars. Group C appears to represent a good compromise for therapy, with substantially higher effect than for the 2 MPa exposures (B, D, and F), but possibly minimizing the excessive impact and large variability seen for group G.

Finally, the lung was found to have injury in some of the rats. Fig. 4d shows a substantial region of pulmonary hemorrhage on the right caudal lung lobe of a sham-exposed rat, which had 10 min of triggered bursts of ultrasound but without contrast agent in the circulation. This lobe was often directly behind the heart and received exposure from the therapy beam, which passed through the heart.

Discussion

High intensity pulsed ultrasound exposure similar to myocardial contrast echocardiography was explored for potential use in tissue reduction therapy in hypertrophic cardiomyopathy. A 1.5 MHz focused transducer was guided by 8 MHz diagnostic images to treat a single

beamline in rat hearts with bursts of 8 pulses. Intermittent pulse bursts with contrast agent microbubbles in the microvasculature induce scattered microlesions of lethally injured cardiomyocyte, which hypothetically could be accumulated in a controlled manner to produce a carefully gauged reduction of a desired tissue volume. Previous work had indicated that bioeffects from contrast aided pulsed ultrasound tended to be self-limiting with the rate of microlesion production rapidly declining with increasing exposure duration. This phenomenon was thought to be a key impediment to MCET. In this study, a base condition of 2 MPa PRPA, 5 cycle pulses and a 1:4 heartbeat trigger interval, which approximated highly efficacious diagnostic conditions, was enhanced by using a higher PRPA of 4 MPa, relatively long pulse-burst intervals of 1:8, and durations of 10 cycles. The ECG was monitored to set the pulse-burst triggering and as a means to monitor treatment progress by observing premature complexes induced by the pulse-bursts. The primary bioeffect was the production of lethally injured cardiomyocytes, assessed by scoring Evans blue stained cardiomyocytes the next day.

The ECG waveform was monitored for indications of heart injury. A few animals had substantial changes, corresponding to substantial heart injury, see Fig. 2. However, the ECG did not reveal consistent changes between pre-, post- and 1-day monitoring periods. In particular, the groups with 2 MPa PRPA had no evident changes in the ECGs indicative of cardiac injury. Results for PCs are listed in Table 1 and showed a wide range of PCs for the different conditions. The increase from 2 to 4 MPa produced significant increases in the fraction of triggered pulse-bursts, which resulted in PCs. The increased trigger interval and pulse duration seemed to increase the bioeffects, but this was not statistically significant. In regard to the expected decline of the rate of PC induction with time, the fraction of PCs at the beginning of exposure was compared to the fraction at the end, as shown in Fig. 3. There were significant reductions in PCs for the three 2 MPa exposure conditions ($P < 0.02$), but not for the 4 MPa exposure conditions ($P > 0.05$). The use of relatively high PRPA therefore appeared to mitigate the saturation phenomenon and allow enhanced efficacy for therapy.

The SCS results are listed in Table 1. For previous work involving diagnostic myocardial contrast echocardiography, the SCS represented a semi-quantitative count of lethally injured cardiomyocytes. For this study, scores were relative and highly variable even within groups. The variability did not appear to be due to initial problems with the scoring, which were corrected. Examples of the distributions of stained cardiomyocytes are shown in Fig. 4. These ranged from a densely scattered array of microlesions along the beam path to infarct-like tissue injury. The reason for the unexpectedly severe injury may be related to the observation by Vancraeynest et al. (2009) that continued myocardial contrast echocardiography-like exposure led to injury similar to ischemia. As for the ECG results, the SCSs were significantly influenced by the PRPA setting but not by the trigger interval or pulse duration. When the SCSs were plotted as a function of the number of PCs in Fig. 4, a definite dose-response appeared to emerge. However, the SCS was highly variable and the linear regression had a poor correlation coefficient of only $r^2 = 0.34$.

The unexpected findings that the longer trigger intervals and pulse duration were not needed to achieve high efficacy were propitious. Overall treatment times can be reduced for shorter trigger intervals, which is clinically advantageous. Shorter pulse durations are also

encouraging because the shorter pulses mean that higher resolution echolocation can be achieved for monitoring lesion production, for example, by passive monitoring of cavitation signals. Therefore, the development of MCET might proceed advantageously toward a combined diagnostic-therapy system, which basically enhances diagnostic myocardial contrast echocardiography to achieve therapeutic efficacy. Finally, the phenomenon of declining microlesion production with time, which led to concern for potentially poor treatment efficacy, proved not to be a problem for higher PRPA therapeutic pulse intensities. Overall, the results indicate that MCET can be refined and potentially lead to a clinically robust therapeutic method for hypertrophic cardiomyopathy and other similar conditions.

Several areas of needed improvement were identified by this study for development of MCET. The use of the ECG for detecting the occurrence of lethal cardiomyocyte injury should be vital for calibrating uncertain exposure levels deep within tissue for the clinical application, and for indicating flagging microlesion production with time. However, each PC seen in the ECG could involve a wide range of microlesion numbers, particularly at the higher exposure levels. The scoring methods should be improved to provide more accurate estimates of microlesion distribution and of total lesion volumes. Some other monitoring method is needed to gauge the density of microlesions in real time, so that efficacy in the range of 20-30% tissue reduction could be achieved and the creation of infarct-like regions of 100% lethality avoided. Once the methods have been refined, more realistic assessment of possible clinical conditions should be tested. For this purpose, a rat model of HCM, such as the congenic salt-sensitive strain (SS-16BN/Mcwi, PhysGen, Medical College of Wisconsin) might be used. This rat model develops cardiac hypertrophy with a left ventricular (LV) wall thickness of about 3 mm, but is not hypertensive (de Resende et al. 2006). Finally, the progression and healing of the treated volume must be followed to demonstrate the actual amounts of tissue reduction obtainable by MCET with minimal perturbation of cardiac function.

Acknowledgments

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

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Figure 1.

Images obtained during alignment and exposure of a rat in group F (reversed right to left). Initial aiming was performed with the focused transducer line-of-sight, which placed the heart at the edge of the sector scan (a). The probe assembly was then shifted to view the heart during exposure (b). The therapy beam then impinged from the side (arrow) to treat the heart through a window between the sternum (S) and lung (L). During treatment the left ventricle and heart tissue filled with microbubbles (c), which were partially destroyed after a pulse-burst (d). The pulse-burst induced a PC in the ECG (arrow).

Figure 2.

Five second ECG records from pre-treatment (a,b,c), post treatment (d,e,f) and the next day (g,h,i), together with heart images made the next day (j,k,l). The right column was from a rat in group A (sham), with no evident change in the ECG or the heart (j). The middle column from a rat in group E with moderate heart injury (k) shows an elevation of the S-T segment (e, arrow) post-exposure that returns to normal by the next day. The right column from a rat in group G with substantial heart injury (l) shows a large elevation in the S-T segment (f, arrow) of the ECG waveform and a persistent T wave inversion (i, arrow) by the next day.

Figure 3.

A plot of the mean fraction with standard error bars of pulse-burst triggers which induced PCs during 1 min periods at the start and near the end of treatment. The groups (B, D, F) with 2 MPa PRPA show a decline in PC induction with time, while the 4 MPa groups (C, E, G) did not.

Figure 4.

Examples of the Evans blue staining of lethally injured cardiomyocytes (scale bars 1 mm) and the pulmonary hemorrhage effect (scale bar 5 mm). Exact positioning of the beam path was not possible for the small hearts. Some hearts had a clear beam path across the entire septum as demarcated by the stained cells (a, group F), while others had the beam limited to the anterior left ventricular wall (b, group B). Several hearts had a substantial infarct-like injury, which exceeded the desired partial myocardial reduction (c, group G). This slice near the apex had some Evans blue containing blood retained in the ventricle. The caudal lobe of the right lung was sometimes hit by the therapy beam, which caused substantial pulmonary hemorrhage (d, group A).

Figure 5.

A plot of the mean stained cell-scores against the mean number PCs for each group with lettered symbol, with standard error bars. There was a weak trend for increasing effect with increasing numbers of PCs as shown by the linear regression against individual rat data with 95% confidence intervals.

Table 1

The seven sets of conditions used for different groups of rats with PC and SCS results (means $\pm$ standard deviation). The abbreviations are: peak rarefactional pressure amplitude (PRPA), pulse average intensity (I_{PA}), pulse duration (PD, using 5 or 10 cycles), trigger interval in number of heartbeats ($\times$ RR), premature complexes (PC in percentage of therapy burst triggers), stained cardiomyocyte score (SCS).

	PRPA	I_{PA}	PD	Interval	PC	Evans Blue
Group	MPa	W/cm ²	μ S	$\times$ RR	% of trig	SCS
A	0	0	-	-	0	9 $\pm$ 9
B	2	173	3.3	4	43 $\pm$ 24	1420 $\pm$ 1493
C	4	892	3.3	4	69 $\pm$ 19	4204 $\pm$ 2777
D	2	173	3.3	8	44 $\pm$ 18	914 $\pm$ 653
E	4	892	3.3	8	78 $\pm$ 6	2782 $\pm$ 1923
F	2	207	6.1	8	63 $\pm$ 7	1745 $\pm$ 1309
G	4	~688	6.2	8	83 $\pm$ 5	6764 $\pm$ 6568