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Are ECG Premature Complexes Induced by Ultrasonic Cavitation Electro-Physiological Responses to Irreversible Cardiomyocyte Injury?

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Abstract

The objective of this study was to explore the relationship between premature complexes in the ECG and lethal injury of cardiomyocytes induced by ultrasound exposure of the heart with contrast-agent gas bodies in the circulation. Anesthetized rats were exposed in a heated water bath to 1.55 MHz focused ultrasound with bursts triggered at end systole during contrast agent infusion. Premature complexes (PCs) were detected in ECG recordings and cardiomyocyte necrosis was scored by identifying Evans blue stained cells in multiple frozen sections. With 0.1 $\mu\text{L/kg/min}$ infusion of contrast agent for 5 min, both effects increased strongly for 2 ms bursts with increasing peak rarefactional pressure amplitude (PRPA) above 1 MPa. At 8 MPa statistically significant effects were found even for no agent infusion relative to sham tests. For 2 ms bursts at 2 MPa, the highly significant bioeffects seen for 10, 1, and 0.1 $\mu\text{L/kg/min}$ infusion became marginally significant for 0.01 $\mu\text{L/kg/min}$, which indicated a lower probability of cavitation nucleation. Burst duration variation from 0.2 to 20 ms produced no substantial trends in the results. Overall, the two effects were well correlated ($r^2=0.88$). The PCs occurring during contrast enhanced ultrasound therefore appear to be electro-physiological responses to irreversible cardiomyocyte injury induced by ultrasonic cavitation.

Keywords

Contrast agent adverse effects; Myocardial Contrast Echocardiography; Arrhythmia; Cardiac Myocyte Necrosis; Gas body activation; Ultrasonic cavitation biology

Introduction

Ultrasound contrast agents consist of suspensions of gas bodies (stabilized gaseous microbubbles) which are designed for maximal persistence in the circulation and strong echo-return in the diagnostic frequency range. The gas bodies, in addition, can serve as near-optimal nuclei for ultrasonic cavitation, a potent mechanism for ultrasonic bioeffects. Commercial and experimental agents have been utilized in numerous bioeffects studies both in vitro and in vivo (Miller et. al. 2008). In the heart, myocardial contrast echocardiography

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(MCE) induced premature complexes (PCs) in the ECG. This effect of myocardial contrast echocardiography has been reported independently (confirming the observation) in humans using experimental contrast agents (van der Wouw et al. 2000; Chapman et al. 2005), in dogs using a commercial agent (Okazaki et al. 2004; Miller et al. 2006), and also in rats with various agents (e. g. Li et al. 2003; Li et al. 2004; Vancraeynest et al. 2009). This bioeffect occurred for intermittent scans (images) at peak rarefactional pressure amplitudes (PRPAs) approaching the upper limit for diagnostic ultrasound, which typically destroyed the optimum gas bodies within the scan plane. The interval between scans was triggered from the ECG to be several heartbeats in duration. This imaging method maximized the image contrast enhancement by allowing refill of the myocardium with blood containing optimum gas bodies between scans. The arrhythmia did not persist for the intervening heartbeats between scans, nor for post-exposure monitoring. The PCs from MCE are important because these provide a clinically observable indication of myocardial perturbation.

Ectopic PC's may arise from many different causal conditions. These include exhaustion and stress, caffeine or alcohol intake and apparently even spontaneously. Many occurrences appear to be benign or simply a nuisance. Mechanical perturbation of the heart can cause PCs by stretch activation, for example during catheterization (Taggart and Sutton, 1991). Heart disease also can lead to arrhythmia, which may not be benign. Ultrasound exposure presents a unique causal condition for PCs, but the exact mechanism remains uncertain. PCs have been reported for cavitation-initiating exposures from lithotripter shockwaves (Delius et al. 1994), as well as from MCE (Miller et al. 2008). However, cavitation activity, as indicated by acoustic emissions, did not necessarily cause PCs (Porter et al. 2001). Indeed, PRPA thresholds for cardiac capillary rupture induced by MCE were less than the threshold for PCs (Li et al. 2003, 2004).

Macroscopic mechanical perturbation by ultrasound has also been implicated in ultrasound perturbation of the heart (Dalecki et al. 1997). A high PRPA focused beam exerts a "radiation force" on tissue, which can produce a mechanical displacement (e. g. see Myers 2006; Melodelima et al. 2007). This tissue average force can be enhanced by the presence of gas bodies (Erpelding et al. 2005). This force could conceivably induce PCs similar to the "touch" induced PCs during catheterization (Taggart and Sutton, 1991).

Myocardial contrast echocardiography also was found to induce lethal cardiomyocyte injury, which was detected by identification of hypercontraction of cardiac myocytes (Miller et al. 2005a) and by staining of dead cardiac myocytes by Evans blue dye (Miller et al. 2005b). The exposure-response trend for the mean PC effect in groups of rats with different PRPA exposure was quite similar to the trend for the mean numbers of necrotic cardiac myocytes found in histology (Miller et al. 2005b). This suggested that lethal microscale perturbation of individual heart cells might have been related to the PC effect, although the correlation between the two bioeffects in individual rats was weak. In vitro studies with cultured heart cells have shown that exposure to ultrasound with contrast agent gas bodies in contact with the cells can elicit membrane depolarization and lead to contraction of the cells (Kudo et al. 2004; Tran et al. 2009). A reversible stretch of the cell membranes likely was responsible for the membrane depolarization in vitro. In vivo, this phenomenon might lead to action potentials and trigger the PCs observed in rats (Tran et al. 2009). The local mechanical stretch of cell membranes by activity of a nearby cavitation site might be lethal or non-lethal. However, the question of whether or not lethal perturbation of the cardiac myocytes was associated with the in vivo PCs was not addressed.

The induction of PCs with contrast agent in the circulation also has been observed with low power burst mode ultrasound, such as is used for physical therapy (Chapman et al. 2005; Dalecki et al. 2005; Rota et al. 2006; Miller et al. 2009). Burst mode exposure with 2 ms

duration bursts at 1.5 MHz with 1:4 triggering produced both PCs and dead cardiomyocytes in histology above an apparent threshold of 0.3 MPa in rats (Miller et al. 2009). The two bioeffects had a statistical correlation coefficient of $r^2 = 0.85$ for data from 30 individual rats. This association between PCs and cardiomyocyte death suggested that ultrasonic cavitation-induced perturbation of cardiomyocytes, particularly the lethal injury, was directly related to the PCs reflected in the ECG.

The objective of this study was to further explore the relation between the cardiomyocyte death and PCs over a much wider range of parameters, than were used before with diagnostic or simulated therapeutic ultrasound, in order to help understand the underlying mechanisms. Relatively high PRPAs up to 8 MPa were used in burst (long pulse) mode ultrasound with a wide range of contrast agent infusion doses. PCs were counted in ECG recordings and cardiomyocyte necrosis was assessed the next day by a new method of scoring Evans blue stained cells in multiple frozen sections covering the scan plane. The results strengthen the association, and are consistent with a casual relationship between lethal cardiomyocyte injury and PCs induced by ultrasound exposure with cavitation nucleation in the myocardium.

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. Hairless rats (CD strain, Charles River, Wilmington, MA, USA) were anesthetized by intraperitoneal (IP) injection of a mixture of ketamine (90 mg kg⁻¹) and xylazine (9 ml kg⁻¹). A 24 gauge cannula was inserted into a tail vein for IV injections. Evans blue dye in saline (20 mg/ml) was injected IP at a dose of 100 mg/kg as a vital stain for cardiomyocytes. 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 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 numbers of normal complexes. The software also partially automated the assessment of PCs, which were seen as single or repeated (re-entry) complexes with a compensatory pause (Miller et al. 2009).

Ultrasound

Ultrasound exposure was provided by a laboratory system with guidance by diagnostic ultrasound imaging, as described previously (Miller et al. 2007, Miller et al. 2009). The laboratory exposure system consisted of a transducer, 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). A 2.54 cm diameter air-backed transducer with 2.54 cm radius of curvature (built in house) was used for high PRPA burst-mode exposures up to 8 MPa. At the focus, the beam had a -6 dB diameter (half power diameter) of 1.7 mm. Four different signals were produced: triggered single bursts of 0.2 ms, 2 ms or 20 ms duration and triggered bursts of bursts with ten 0.2 ms bursts spaced 2 ms apart (20 ms duration). The triggering was adjusted from the ECG displayed on the oscilloscope to occur at end-systole every four cardiac cycles. The PRPA of

each signal was measured with a calibrated hydrophone (model 805, Sonora Medical Systems Inc., Longmont CO USA). All PRPA values specified are the measured free-field water values. The attenuation by the chest wall at 1.5 MHz was previously found to be minimal at about -1.2 dB (for diagnostic ultrasound pulses, Miller et al. 2005b).

For exposure, a diagnostic ultrasound machine (GE Vingmed System V, General Electric Co., Cincinnati OH USA) was used to image the heart with an FPA10 probe. Once a clear image of the left ventricle was obtained at the level of the papillary muscles, the single-element exposure transducer was moved into the same aiming position, which provided a clear and reproducible targeting of the rat heart at the focal zone. Definity® (Lantheus Medical Imaging, Inc., N. Billerica, MA) ultrasound contrast agent was diluted in sterile saline and infused via tail vein at 0.5 ml/kg/min of diluted agent. After aiming, the ECG was recorded for 5 min pre-exposure, 5 min during exposure with infusion of diluted contrast agent for most tests, and 5 min post-infusion with continued exposure.

The infusion of contrast agent produces a rapid increase in circulating gas bodies to a plateau, and a rapid decline to zero after infusion (Miller et al. 2010). For one group in this study with an infusion dose rate of 10 μ l/kg/min, the circulating contrast agent dose was monitored in the left kidney by generating time-intensity curves for image brightness, as described previously (Miller et al. 2010). For this purpose, a Powervision 8000 ultrasound machine (model SSA-390A, Toshiba Corporation, Tochigi-Ken, JPN) with curved linear array (PVN-375AT) was used for flash echo imaging with 10 sec interval. Curves were acquired twice for each rat in this group, once for infusion without exposure of the heart, and again (after a delay of 5 min) with exposure of the heart. This procedure provided exponential decay time-constants after infusion, and an estimate of the reduction in circulating dose caused by gas body destruction by the ultrasound exposure of the heart.

Cardiomyocyte scoring

Lethal injury of cardiomyocytes was scored on the basis of Evans blue staining, essentially as described previously (Miller et al. 2005b), but multiple sections were examined to search for stained cells. The hearts were removed the day after exposure and cleared of most blood with heparin-saline. In the previous work (Miller et al. 2005b), only one section from the center of the scan plane, which appeared to have a maximal number of stained cells, was scored for each heart sample. In the present study, most samples had no clear external indication of the location of exposure. Therefore, each heart was sectioned to provide samples covering almost the entire exposed myocardial volume. The new method was used to search for low numbers of stained cells, which might occur throughout the ultrasound beam, and thus provide a more sensitive evaluation. To search for stained cardiomyocytes, the apex of the heart was cut away to the bottom of the left ventricle. Then, 10 μ m thick sections were cut each 200 μ m into the sample to provide 30 slides 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 cell score (SCS). This provided nominal coverage over about 6 mm of the heart. However, because the cardiomyocytes were about 16 μ m in diameter and 128 μ m in length (Gerdes et al. 1986), this procedure provided an under-estimate of the total number of stained cells, which would be at least twice as great.

Experimental Plan

The study was conducted in 14 groups of 5 rats each and one group (#6) with 4 rats as listed in Table 1. Sham control tests included infusion of the saline vehicle for contrast infusion and infusion of agent at 10 μ l/kg/min without ultrasound exposure. A PRPA-response test was conducted for 0.1 μ l/kg/min infusion and 2 ms bursts for groups with 1, 2, 4 and 8 MPa exposure. The microscale cavitation-induced perturbation and the radiation force-induced

tissue displacement were expected to increase strongly with PRPA. A gas body dose-response test was conducted for 2 MPa exposure and 2 ms bursts for groups with 0.01, 0.1, 1 and 10 $\mu\text{l/kg/min}$ infusion. The radiation force mechanism and the number of sites of microscale cavitation perturbation were expected to increase roughly in proportion to the infused dose. Three groups were exposed at 8 MPa with no infusion using 2 ms or 20 ms bursts, or with infusion of the saline diluent vehicle using 2 ms bursts. These groups were intended to assess possible radiation force-induced induction of PCs without added contrast agent. Finally, groups with 2 MPa exposure and 0.1 $\mu\text{l/kg/min}$ infusion were tested for 0.2 ms, 2 ms or 20 ms bursts, and for ten 0.2 ms bursts spaced 2 ms apart (total burst of bursts duration of 20 ms). For these conditions, the micro-scale cavitation activity was expected to be similar, while the radiation force-induced tissue displacement was expected to vary with burst duration.

Data were collected for each rat on PCs in the ECG recording and for SCSs in the frozen sections. The PCs were converted to a percentage of the total number of scan triggers, which varied with heartbeat for the timed exposures. Results are reported as the means plus or minus one standard deviation, or plotted with standard error bars. Statistical analysis was performed using SigmaPlot for Windows V. 11.0 (Systat Software Inc., San Jose CA, USA). Student's t-tests or the Mann-Whitney rank sum test, as appropriate, were used to compare means of the measured parameters, with statistical significance assumed at $P < 0.05$. For multiple pair-wise comparisons, the Student-Newman-Keuls method was used to correct the statistical significance tests. The nonlinear regression function was used to fit exponential rise to maximum and exponential decay curves to some data sets.

Results

A heart exposed to 2 MPa with 10 $\mu\text{l/kg/min}$ infusion (Group 12) is shown in Fig. 1. A circular collection of blue-stained cells was visible on the anterior surface, which marks the entrance of the focused beam (Fig. 1a). On the posterior side of the heart (Fig. 1b), an exit mark is also visible with scattered stained cells at a higher position on the heart (i. e. the beam passed through the heart at an angle relative to a direct cross-section). For the other exposure conditions, which had lower doses of the contrast agent, there were rarely any clearly visible external indications of cell staining. For all samples, the entire central portion of the heart, which presumably included both entry and exit positions, was sampled as describe in Methods. A display of the scores for the thirty slides scored for each of the 5 rats in group 12 with 2 MPa exposure and 10 $\mu\text{l/kg/min}$ infusion is shown in Figure 2a. For comparison, the scores for group 7 with 2 MPa with 0.1 $\mu\text{l/kg/min}$ infusion is shown in Fig. 2b, which are greatly reduced from the results in Fig. 2a.

The relative concentration of gas bodies in the circulation was assessed by measuring time-intensity curves in kidney tissue (Miller et al. 2010), without and with exposure of the heart for rats in group 12. Group 12 with 10 $\mu\text{l/kg/min}$ infusion had substantial brightening upon infusion (wash-in) which decayed after infusion (wash-out) in the right kidney. The plateau value was significantly reduced when the heart was exposed, from 12.6 ± 9.6 relative units without to 4.2 ± 4.4 relative units ($P < 0.05$ for a paired t-test) with exposure of the heart. Post-infusion decay constants were 89.7 ± 30.1 s without and 23.6 ± 10.1 s with exposure ($P < 0.01$). This behavior was quite similar to the results described previously for monitoring of the left kidney and exposure of the right kidney (Miller et al. 2010), and indicated substantial destruction of gas bodies in the ventricles and scanned tissue during exposure.

Results for PCs and stained cells in the 15 groups are listed in Table 1. Increases in PRPA produced increases in PCs to a plateau approaching 80% of the possible total (the number of triggers) as shown in Fig. 3. The stained cell score increased monotonically to scores

representing thousands of cells at 4 and 8 MPa with the 0.1 $\mu\text{l/kg/min}$ infusion with both bioeffects significantly different from shams (group 2) for 2, 4 and 8 MPa. The 1 MPa group (#6) had significantly increased stained cell scores ($P<0.05$) but not PCs relative to the sham (group 2).

Two groups were exposed at 8 MPa with no gas body infusion for 2 ms (group 3) and 20 ms (group 4) bursts. The 2 ms group had significantly increased PCs relative to shams ($P<0.01$) and the 20 ms group was further increased ($P<0.02$). However, both groups had variable SCSs, which were larger but not significantly different from the sham group. Another group (5) had vehicle infusion with 2 ms bursts at 8 MPa, with no significant difference relative to the no infusion group (3) for either effect. These results suggested that the 8 MPa exposure without gas body infusion may have been sufficient to induce detectable cavitation bioeffects, but with large standard deviations. If the two sham groups (1 and 2) results were combined and compared to the two 8 MPa groups (3 and 5) to increase statistical power, then both effects were statistically significant ($P<0.01$ for PCs and $P<0.05$ for SCSs) for the larger groups ($n=10$) using the Mann-Whitney rank sum test.

Increases in the infusion dose rate produced comparable increases in both bioeffects for 2 MPa exposure with 2 ms bursts as shown in Fig. 4. Again, as for PRPA increases, the PCs tended to approach a plateau as the maximum 100% of triggers was approached. Both results were highly significant for 0.1 $\mu\text{l/kg/min}$ and greater infusion rates. For the 0.01 $\mu\text{l/kg/min}$ infusion, both bioeffects seemed to occur in a hit or miss fashion but were not statistically significant.

The burst duration variations for 2 MPa exposure with 0.1 $\mu\text{l/kg/min}$ infusion were highly significantly different from the sham (group 2), as shown in Fig. 5. Although the SCS seemed to increase with increasing burst duration, there were no significant differences in the either effect for the different burst durations. The virtual equivalence of result for the burst of 10 bursts of 0.2 ms duration to the result for the single 0.2 ms burst was typical of effects for contrast enhanced ultrasound, because the gas bodies apparently were destroyed at the first burst and give no additional effect for the other 9 bursts.

For all the exposure conditions tested, PCs were accompanied by the detection of vital-stained cardiomyocytes the next day. This observation supports the idea that the lethal cell injury was directly related to the PCs. Figure 6 is a plot of the PC results versus the stained cell scores for all the exposures ($n=65$). The numbers of PCs increased rapidly and then level off for increases SCS. The fitted curve ($r^2=0.88$) is an exponential rise to maximum for which the maximum was 253 PCs (about 80% of the burst triggers). The initial linear portion indicates 0.22 PCs per unit SCS. That is, an SCS increment of about 4.5 was found for each PC. Given the under-counting of stained cells (see Methods), 10 or more cells may have been lethally injured for each observed PC.

Discussion

This study explored the relationship between PCs appearing in the ECG and the detection of lethally injured cardiomyocytes for ultrasound exposure with contrast agent gas bodies in the circulation. A possible connection between PCs and lethal cell injury has been noted previously (Miller et al. 2005b; Miller et al. 2009). In this study, data was accumulated to help discriminate between the possible operative PC-induction mechanisms of macroscopic radiation force with tissue displacement and of microscopic perturbation of cardiac myocytes at cavitation nucleation sites. Furthermore, the possible induction of PCs by non-lethal perturbation of the cardiac myocytes was considered in addition the lethal injury. New methods were used to provide a sensitive assessment of the number of lethally injured cells

in an exposed heart as a stained cell score (SCS). Examples of the observed effect and the distribution of stained cells over 30 slides scored for each heart are shown in Fig. 1 and 2, respectively.

The incidence of both bioeffects increased rapidly with PRPA for 0.1 $\mu\text{l/kg/min}$ Definity infusion above 1.0 MPa (Fig. 3). The infusion was substantially less than the infusion rate recommended for diagnostic ultrasound and illustrated the cavitation nucleation ability of the contrast agent gas bodies. Both effects increased as expected for the microscopic cavitation mechanism of cardiac myocyte perturbation. The observation that both the PCs and SCS had a threshold of about 1 MPa suggest that the PCs were associated directly with the lethal cardiomyocyte injury, because the PCs would be expected to occur below the threshold for lethal injury if sub-lethal injury was an important origin of the PCs.

Sham (0 MPa) exposure with infusion of the sterile saline vehicle or 10 $\mu\text{l/kg/min}$ contrast agent had non-zero stained cell scores (Table 1). A low incidence of necrotic cells is expected due to normal cell turnover (Buja and Vela, 2008), and the detection of these cells reflects the relatively high sensitivity of the method. One PC was recorded for the 10 $\mu\text{l/kg/min}$ sham group. At the highest PRPA of 8 MPa, a significant increase in both the bioeffects was found for the combined groups without the addition of contrast agent gas bodies. The detection of stained cells suggests that cell killing rather than the macroscopic radiation force was the mechanism of action for the PCs, because the possible radiation force induced PCs did not occur in the absence of increased cell killing. The occurrence of cavitation bioeffects for 8 MPa PRPA at scattered nucleation sites implies the existence of sub-optimal cavitation nuclei in normal tissue which can be activated at PRPAs achieved with high intensity focused ultrasound (HIFU). This result is comparable to other observations in muscle (Hynynen, 1991). The existence of suboptimal nuclei in normal tissue is also evident from histotripsy research, for which cavitation is initiated and controlled for tissue ablation (Xu et al. 2008).

The incidence of both bioeffects also increased rapidly for infusion dose rates above the lowest 0.01 $\mu\text{l/kg/min}$ rate (Fig. 4), for which the results were variable (hit or miss) and not statistically significant. The enhancement of the macroscopic radiation force and displacement of tissue did not appear to have an important role in the PC effect, because the combined induction of PCs and a SCS was consistent with a cardiomyocyte perturbation mechanism for the PCs. In addition, stretch-induced induction of PCs would be expected to have a sigmoidal response over a narrow range of stretch (Franz et al. 1992), but, in fact, the PCs occurred incrementally over a 1000-fold change in dose of contrast agent gas bodies.

The concentration of effective gas bodies (at 2 MPa) in the myocardial tissue can be estimated for the threshold-like infusion rate of 0.01 $\mu\text{l/kg/min}$. A estimate of the circulating dose can be calculated by multiplying the decay constant times the total dose, divided by the total infusion time (Miller et al. 2010). From the time-intensity curves described above, the total dose of 50 nl/kg over 300 s gave a circulating dose of about 4 nl/kg for the exposures with a 23.6 s decay constant. There were about 6.9×10^9 gas bodies per ml of contrast agent, which includes losses in handling and dilution (Li et al. 2004). This concentration indicates a dose of about 27,600 gas bodies per kg. Since a rat has about 64 ml/kg of blood (Lee and Blafox, 1985), this dose implies a concentration of 431 gas bodies per ml of blood. The focal zone had a -6 dB diameter of 1.7 mm and passed through about 7 mm of myocardium (omitting about 4 mm of blood-filled path length in the ventricles), which indicates that about 16 μl of myocardium was exposed in the focal zone. The percentage of capillary volume for rat myocardium has been reported to be 9.2 % on average (Hossler et al. 1986), giving approximately 1.5 μl of capillary blood exposed in the focal zone. Within this volume of circulating blood, there should have been about 0.65 gas bodies (1.5 μl times 0.43 gas

bodies per μl), which refilled for each triggered exposure burst. Given that not all gas bodies would be suitable for cavitation nucleation and lethal cardiomyocyte injury for the 2 MPa PRPA, the hit-or-miss results at 0.01 $\mu\text{l/kg/min}$ were likely due to a hit-or-miss population of optimal gas bodies in myocardial capillaries within the focal zone.

The radiation force mechanism for PCs was expected to be dependent directly on the duration of ultrasound bursts, with longer bursts imparting a greater “push” on the tissue. For the conditions used in this study, this does not appear to be the acting mechanism, as shown in Fig. 5. Pulse durations from 0.2 ms to 20 ms all had about the same PC effect, as did a burst of ten 0.2 ms bursts. This behavior is characteristic of cavitation bioeffects, for which local cell interactions occur within a few ultrasound cycles, and again disfavors a causative role for a tissue average interaction in cavitation induced PCs.

The mechanism by which ultrasound exposure of the myocardium induces PCs appears to be microscopic-cavitation interaction with cardiomyocytes. The occurrence of PCs has been associated with cavitation emissions (Rota et al. 2006). The good correlation (Fig. 6) of lethally injured cardiomyocytes with the PCs supports the hypothesis that the lethal injury was directly related to the PCs. A cause-and-effect relationship seems plausible due to the close temporal association of the PCs with ultrasound exposure, and the hyper-contracted state of scattered cardiomyocytes found immediately after exposure (Miller et al. 2005a). Cardiomyocytes can transmit the contractile stimulus end-to-end via gap junctions in the intercalated discs (Berne and Levy, 2001), including hypercontraction (contraction band necrosis) under some conditions (Garcia-Dorado et al. 2004). One, or a small local group of lethally injured cardiomyocytes undergoing hypercontraction at the cavitation site could become the central trigger for an ectopic depolarization.

The possibility of the induction of some PCs by sub-lethal interaction with cardiomyocytes cannot be ruled out. However, the association between PCs and the SCS was consistent for diagnostic and therapeutic ultrasound with contrast agent and even for HIFU without added contrast agent gas bodies. PCs had the same PRPA threshold and faded away at the same low agent dose as the SCS. The occurrence of PCs during contrast-enhanced ultrasound therefore represents a useful electro-physiological response to irreversible cardiomyocyte injury and necrosis induced by ultrasonic cavitation. In regard to clinical myocardial contrast echocardiography, this link affords the clinician a simple safety strategy. Lethal cardiomyocyte injury apparently can be avoided simply by reducing the PRPA (power level or Mechanical Index on ultrasound machines) if PCs appear in the ECG.

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Figure 1. Photomicrographs of (a) the anterior view and (b) posterior view of a rat heart exposed to 2 ms bursts of 2 MPa with 10 μ l/kg/min contrast agent infusion. The circles (4 mm diam) indicate the beam entry and exit regions marked by staining of cardiomyocytes.

Figure 2.

Plots of the stained cell scores for each of 30 slides for (a) the hearts in group 12 with 10 µl/kg/min contrast agent infusion, for which the open symbols represent the heart shown in Fig. 1, and (b) the hearts in group 7 with 0.1 µl/kg/min contrast agent infusion.

Figure 3.

Exposure-response trends for the stained cell scores (top) and PCs (bottom), which were expressed as a percentage of the number of bursts triggered during the exposure for 2 ms bursts and 0.1 $\mu\text{l/kg/min}$ infusion. All the exposed-sample results were increased significantly from the shams (*), except for the PCs at 1 MPa.

Figure 4. Contrast agent dose-response trends for the stained cell scores (top) and PCs (bottom), expressed as a percentage of the number of bursts triggered during the exposure for 2 ms bursts at 2 MPa. All the exposed-sample results were increased significantly from the shams (*), except for the 0.01 μl/kg/min infusion.

Figure 5. Results for the stained cell scores (top) and PCs (bottom) found for the different burst durations, and for a burst of ten 0.2 ms bursts. All the exposed sample results were increased significantly from the shams (*).

Figure 6. Stained cell scores and PC results for the 65 individual rats which received ultrasound exposure with and without contrast agent infusion. The non-linear fitted curve is an exponential rise to a maximum of 253 (about 81 % of scan triggers) PCs.

Table 1

A list of the test groups and results (means $\pm$ standard deviation) for premature complexes (PCs) and stained cell score (SCS).

#	Condition			Results		
	Burst Dur. ms	PRPA MPa	Infusion μ l/kg/min	PCs percent	Total SCS	
1	0	0	0 (vehicle)	0 $\pm$ 0	17.4 $\pm$ 27.9	
2	0	0	10	0.06 $\pm$ 0.13	11.4 $\pm$ 8.6	
3	2	8	0	0.74 $\pm$ 0.57	85.8 $\pm$ 145	
4	20	8	0	4.3 $\pm$ 3.4	86.5 $\pm$ 87.5	
5	2	8	0 (vehicle)	0.34 $\pm$ 0.49	65.0 $\pm$ 82.7	
6	2	1	0.1	0.25 $\pm$ 0.17	81.5 $\pm$ 63.8	
7	2	2	0.1	18.3 $\pm$ 6.3	356 $\pm$ 210	
8	2	4	0.1	73.0 $\pm$ 15.9	1719 $\pm$ 1169	
9	2	8	0.1	70.4 $\pm$ 25.4	2587 $\pm$ 2182	
10	2	2	0.01	0.96 $\pm$ 0.94	72.4 $\pm$ 76.7	
11	2	2	1.0	63.2 $\pm$ 10.8	2166 $\pm$ 952	
12	2	2	10	73.9 $\pm$ 7.9	8711 $\pm$ 2529	
13	0.2	2	0.1	10.6 $\pm$ 3.3	153 $\pm$ 120	
14	20	2	0.1	12.7 $\pm$ 8.2	320 $\pm$ 327	
15	0.2 $\times$ 10	2	0.1	14.0 $\pm$ 6.8	276 $\pm$ 188	