CONTRAST AGENT-INDUCED CARDIAC ARRHYTHMIAS IN RATS

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Abstract - Although no known meaningful health risks have been reported following the use of microbubble ultrasound echogenic contrast imaging agents (MUECIA) in the United States, premature ventricular contractions have been documented in a report from the Netherlands and anecdotally noted in clinical use. Based on this information, we designed a study to characterize electrocardiogram conduction abnormalities in rat hearts exposed to pulsed ultrasound following injection of contrast agent. A focused 51-mm-diameter, 3.1-MHz, lithium niobate transducer that had *in situ* (at the heart) peak rarefactional and compressional pressures of 15.9 MPa and 36.1 MPa, respectively, (1.7-kHz PRF and 1.3-μs pulse duration) was used to expose each heart. Twenty rats were anesthetized, clipped, depilated, and connected to a digital ECG recorder. Electrocardiograms were recorded digitally and continuously during seven experimental intervals: ECG baseline; exposure to pulsed ultrasound without intravenous MUECIA; discontinue ultrasound exposure and then inject intravenous MUECIA; expose to pulsed ultrasound with intravenous MUECIA; discontinue ultrasound exposure; exposure to pulsed ultrasound with intravenous MUECIA; and discontinue ultrasound exposure. Rats had no meaningful conduction abnormalities when exposed to ultrasound alone or contrast agent alone but developed premature atrial (PAC) and ventricular complexes (PVC) and polymorphic ventricular tachycardia (PVT) when contrast agent was administered intravenously and the heart exposed to pulsed ultrasound. When ultrasound exposure was concluded, cardiac arrhythmias ceased but reoccurred when ultrasound exposure was resumed. Necrotic cardiac rhabdomyocytes were observed microscopically in myocardium of rats exposed to ultrasound following injection of contrast agent. Because cardiac arrhythmias were induced only when contrast agent interacted with ultrasound during exposure, the presence of myocardial lesions alone did not appear sufficient to serve as foci for ectopic electrical activity. These results suggest that microbubble contrast agent through its biomechanical interactions with pulsed ultrasound has the potential to induce conduction abnormalities leading to potentially hazardous cardiac arrhythmias.

I. INTRODUCTION

Diagnostic (pulsed) ultrasound is one of the most widely used and safest imaging modalities available in medical practice today [1]. To improve the imaging capabilities of diagnostic ultrasound systems and ultrasound examinations, MUECIA are being approved by the FDA and introduced into the United States health care market. MUECIA, when administered intravenously, are effective because they substantially enhance the contrast between tissues with similar acoustic impedance such as ventricular myocardium and the blood within the ventricle. Contrast enhancement results from increased reflection of ultrasound from microbubbles circulating in blood vessels, vascular beds, and heart chambers when compared to adjacent tissue. MUECIA are used as adjuncts in routine ultrasound evaluations to enhance sonographic contrast and thus increase the opportunity for early detection, diagnosis, and treatment of a variety of disease processes including heart disease and cancer.

Premature ventricular contractions/complexes have been reported in healthy adult human beings during triggered second harmonic imaging of a contrast agent for myocardial perfusion [2]. In addition, anecdotal

reports of premature contractions associated with the use of MUECIA have been described in medical practice.

The purpose of this study was to determine if cardiac arrhythmias could be induced in rats injected with MUECIA and exposed to pulsed ultrasound.

II. MATERIALS AND METHODS

Animals

The hearts of twenty 10- to 11-week-old 269±12g female Sprague-Dawley rats (Harlan, Indianapolis, IN) were exposed to pulsed ultrasound. Rats were weighed and anesthetized with ketamine hydrochloride (87.0 mg/kg) and xylazine (13.0 mg/kg) administered intraperitoneally. The skin over the sternum was exposed by removing the hair with an electric clipper, followed by a depilatory agent to maximize sound transmission. An intravenous catheter with a 25-gauge needle was inserted and taped into the lateral tail vein.

A black dot was placed on the skin over the sternum at approximately 1.7-cm cranial to the xyphoid process to guide the positioning of the transducer's ultrasonic beam. Anesthetized rats were placed in dorsal recumbancy. A stand off tank was positioned in contact with the skin using mineral oil as a coupling agent. The transducer, placed in the stand off tank that contained degassed water at 30°C, was aligned with the black dot.

The low-power pulse-echo capability of the exposure system (RAM 5000, Ritec, Inc., Warwick, RI), displayed on a digital oscilloscope (LeCroy Model 9354CTM, Chestnut Ridge, NY), was used to focus the calibrated transducer's focal region on the heart.

The experimental protocol was approved by the campus' Laboratory Animal Care Advisory Committee.

Electrocardiography

ECG data were collected continuously from each rat during the entire duration of the experimental period. The experimental period was divided into 7 timed intervals with each interval serving as a control for the subsequent interval. ECG electrodes were inserted into the skin of the limbs to produce a lead configuration analogous to human ECG lead I (left front limb - positive, right front limb - negative, right rear limb – ground). ECGs were recorded digitally using a SRA-400b Sinus Rhythm Analyzer (Micro-Med Inc., Louisville, KY).

The first interval was used to collect baseline ECG data (normal sinus rhythm) on anesthetized rats prior to intravenous administration of MUECIA and/or exposure to ultrasound. The second interval was used to collect ECG data on anesthetized rats exposed to ultrasound prior to the intravenous administration of MUECIA. During the third interval, 0.25 ml of Optison® was injected intravenously. During the fourth interval, the heart was exposed to pulsed ultrasound while MUECIA was circulating in the cardiovascular system. During the fifth interval, ultrasound exposure was discontinued. During the sixth interval, the heart was exposed to pulsed ultrasound while MUECIA continued to circulate in the cardiovascular system. During the seventh interval, ultrasound exposure was discontinued.

The character and type of conduction abnormalities induced in each rat were determined and then confirmed with comparisons to ECG abnormalities demonstrated in standard medical textbooks [3].

Pathologic Examination

Each rat was killed immediately following termination of ECG recording by cervical dislocation while under anesthesia. The heart from each rat was examined for macroscopic lesions and then fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for routine microscopic evaluation.

Exposimetry

Exposure conditions and transducer calibration procedures used in this study have been described previously [4,5]. A focused 51-mm-diameter, lithium niobate ultrasonic transducer (Valpey Fisher, Hopkinton, MA) was characterized in water to have a center frequency of 3.1 MHz, a fractional bandwidth of 15%, a focal length of 56 mm, a -6-dB focal beamwidth of 610 μm, and a -6-dB depth of focus of 5.9 mm. The calibration procedure yielded an *in vitro* peak rarefactional pressure of 20.2 MPa, an *in vitro* peak compressional pressure of 45.8 MPa, a Mechanical Index of 5.8, and a pulse duration of 1.3 μs. The *in situ* (in the heart) acoustic pressures were estimated by taking into account the ultrasonic attenuation of the interposed tissue, as measured previously [6], to yield at

the center of the focal region (6 mm from the skin surface) an *in situ* peak rarefactional pressure of 15.9 MPa and an *in situ* peak compressional pressure was 36.1 MPa. The exposure of the rat's heart was conducted at a pulse repetition frequency of 1700 Hz.

III. RESULTS

Electrocardiography

The sum of the number of conduction abnormalities induced in each exposure interval is summarized in **Figure 1** for the 20 rats.

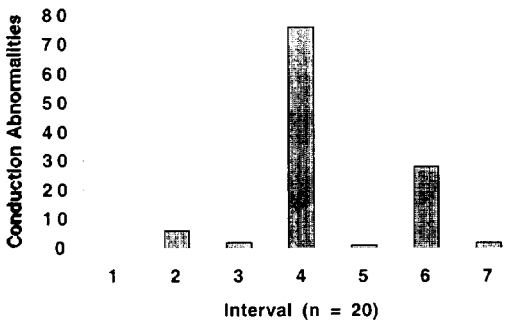

Figure 1: Number of conduction abnormalities induced in each exposure interval.

No conduction abnormalities were observed during interval 1 (normal sinus rhythm under anesthesia). Occasional single premature contractions were observed immediately at the onset of interval 2 when rats were exposed to ultrasound alone without intravenous administration of MUECIA. After the initial premature contractions, the ECG complex configuration returned to normal (sinus rhythm).

During interval 3, when MUECIA was injected intravenously, rare single premature contractions were observed. When rats were exposed to pulsed ultrasound following intravenous injection of MUECIA (interval 4), cardiac arrhythmias were induced immediately when exposure was initiated and continued for the entire exposure interval.

These cardiac arrhythmias included sustained episodes of ventricular tachycardia and premature atrial and ventricular contractions (predominately a polymorphic configuration) (**Figure 2**). After the ultrasound exposure interval was concluded, the configuration of the conduction complexes rapidly returned to normal (sinus rhythm). When each rat was exposed for a second time to pulsed ultrasound (interval 6), ventricular tachycardia and premature contractions resumed but were observed less frequently when compared to the number that occurred in interval 4. During interval 7, when ultrasound exposure was terminated, ventricular tachycardia ceased but occasional premature contractions occurred.

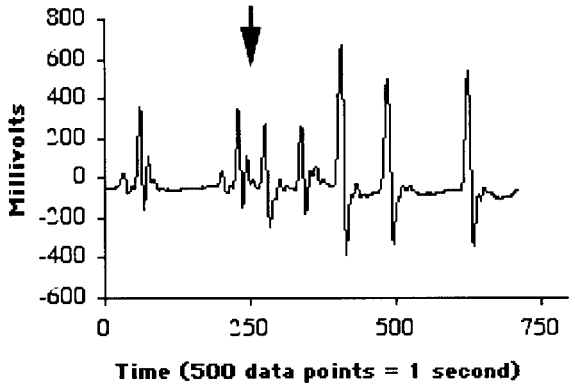

Figure 2: Premature ventricular contractions induced in a rat injected with contrast agent and exposed to pulsed ultrasound (arrow).

Pathologic findings

One rat had macroscopic lesions of hemorrhage and tissue necrosis in the right ventricular free wall. There were no visible lesions in the interventricular septum or free wall of the left ventricle.

Microscopic lesions consisted of one or more foci of swollen, hyalanized necrotic rhabdomyocytes without an inflammatory response (acute coagulative necrosis) (**Figure 3**). No hemorrhage was observed with these lesions. In general, the location of the lesions corresponded to the path of the ultrasound beam; however, lesions were scattered at random within the field. Occasional lesions were located under the epicardium. Lesions were present within the right ventricular free wall, interventricular septum, and left ventricular free wall.

IV. CONCLUSIONS

Under the conditions used in this experiment, we have demonstrated that the use of a MUECIA was associated with atrial and ventricular premature contractions and polymorphic ventricular arrhythmias when the heart was exposed to pulsed ultrasound. There were no meaningful conduction abnormalities

when the heart was exposed to pulsed ultrasound alone or when exposed to MUECIA alone.

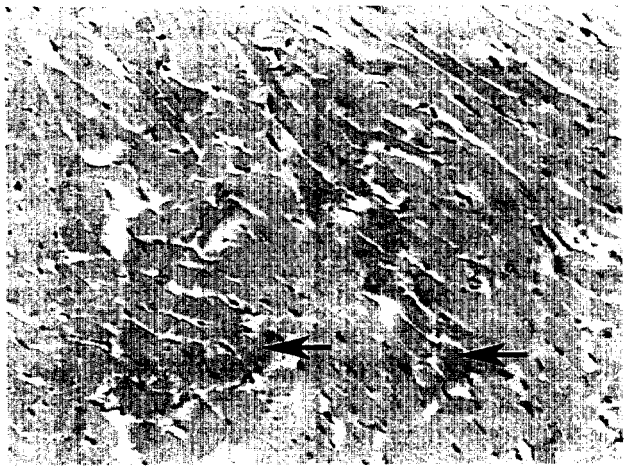

Figure 3: Heart muscle from a rat injected with contrast agent and exposed to pulsed ultrasound. Areas of tissue injury are indicated by arrows; however, gray scale images fail to show subtle changes in tissue color indicative of injury.

The character and location of rhabdomyocyte lesions were appropriate for the genesis of ectopic electrical signals that could lead to ventricular arrhythmias. The finding that ventricular arrhythmias in rats injected intravenously with MUECIA ceased when ultrasound exposure was terminated suggests that rhabdomyocyte injury in itself was not sufficient to induce arrhythmias. It is plausible that the interaction of MUECIA with an ultrasound field resulted in biomechanical phenomena that lead to electrical instability. Electrical instability ceased when ultrasound exposure ended.

Of the known biomechanical phenomena associated with the interaction of MUECIA with an ultrasound field, inertial cavitation is the most plausible mechanism that could lead to the development of ventricular arrhythmias [7]. The observation that ventricular arrhythmias ceased when ultrasound exposure ended suggested that tissue injury and death alone were not sufficient contributors to the mechanism that resulted in ectopic electrical foci.

V. ACKNOWLEDGEMENTS

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