Destruction of Contrast Microbubbles by Ultrasound
Effects on Myocardial Function, Coronary Perfusion Pressure, and Microvascular Integrity

Taniyel Ay, MD; Xavier Havaux, MS; Guy Van Camp, MD; Barbara Campanelli, MD; Giovanna Gisellu; Agnès Pasquet, MD; Jean-François Denef, MD; Jacques A. Melin, MD, PhD; Jean-Louis J. Vanoverschelde, MD, PhD

Background—Recent experimental data indicate that ultrasound-induced destruction of ultrasound contrast microbubbles can cause immediate rupture of the microvessels in which these microbubbles are located.

Methods and Results—To examine the functional and morphological significance of these findings in the heart, isolated rabbit hearts were perfused retrogradely with buffer containing ultrasound contrast agents and were insonated at increasing levels of acoustic energy with a broadband transducer emitting at 1.8 MHz and receiving at 3.6 MHz and operated in the triggered mode (1 Hz). At the end of each experiment, the hearts were fixed in glutaraldehyde and examined with light microscopy. Neither exposure to ultrasound alone or to contrast alone affected left ventricular developed pressure. By contrast, simultaneous exposure to contrast and ultrasound resulted in a reversible, transient mechanical index (MI)–dependent decrease in left ventricular developed pressure (to 83±5% of baseline at an MI of 1.6) and a transient MI-dependent increase in coronary perfusion pressure (to 120±6% of baseline at an MI of 1.6). Myocardial lactate release also showed significant increases with increasing MIs. Macroscopically, areas of intramural hemorrhage were identified over the beam elevation in hearts exposed to both contrast and high-MI ultrasound. Light microscopy revealed the presence of capillary ruptures, erythrocyte extravasation, and endothelial cell damage. The mean percentage of capillaries ruptured at an MI of 1.6 was 3.6±1.4%.

Conclusions—Simultaneous exposure of isolated rabbit hearts to ultrasound and contrast agents results in an MI-dependent, transient depression of left ventricular contractile function, a rise in coronary perfusion pressure, an increase in lactate production, and limited capillary ruptures. (Circulation. 2001;104:461-466.)

Key Words: contrast media ▪ echocardiography

Myocardial contrast echocardiography (MCE) is a relatively new technique that uses ultrasound contrast agents to produce myocardial opacification and assess myocardial perfusion. The ultrasound contrast agents used to produce myocardial opacification typically consist of gas microbubbles <10 μm in diameter that cross the pulmonary capillary bed and persist long enough to reach the myocardium. Because of their acoustic properties, these microbubbles considerably enhance the backscattering capabilities of blood, thereby allowing the myocardial capillary bed to be imaged with ultrasound.

It is now well established that contrast microbubbles can be destroyed by acoustic pulses used in the frequency and intensity range of diagnostic cardiac ultrasound.1,2 The process by which ultrasound destroys microbubbles has been characterized previously.3 Because microbubbles are compressible, they alternately contract and expand in the acoustic field, a phenomenon often referred to as cavitation. At low acoustic pressure, microbubbles usually grow and shrink rhythmically and symmetrically around their equilibrium size. This phenomenon is known as stable or noninertial cavitation. At higher acoustic pressure, however, the expansion and contraction of the microbubbles usually become unequal and markedly exaggerated, leading to their destruction. This second form of activity is known as inertial cavitation. There is accumulating evidence that the process of cavitation, particularly inertial cavitation, may produce capillary damage to organs that contain air, such as the lungs,4 or that are exposed to ultrasound contrast agents, such as has been demonstrated recently in rat spinotrapezius muscle.2,5 The purpose of the present study was to determine whether capillary damage...
also occurs in hearts exposed to ultrasound contrast agents and to evaluate the potential consequences thereof on left ventricular (LV) contractile performance.

Methods

Isolated Hearts
Hearts were removed from 1.5- to 2.0-kg New Zealand White rabbits, perfused retrogradely at 37°C with nonrecirculating erythrocyte-enriched Krebs-Henseleit buffer, and paced at 180 bpm as described previously.6 Red blood cell concentration was adjusted to obtain a hematocrit of 25%. The perfusate was equilibrated with a mixture of room air and 5% CO2 to maintain perfusate PO2 and pH within the physiological range. LV pressure was measured with a compliant latex balloon placed in the LV cavity via the left atrium and connected to a P23Db pressure gauge transducer (Gould Laboratories).

Myocardial Contrast Echocardiography
PESDA (perfluorocarbon-enhanced sonicated dextrose albumin), a second-generation contrast agent, consisting of decafluorobutane-filled albumin microbubbles with a mean diameter of 4.2±0.5 μm and a mean concentration of 1010·mL−1, was used in the present study.7 Experiments were also conducted with the following manufactured contrast agents: Sonozoid (Nycomed Amersham), Optison (Mallinckrodt), and Levovist (Shering AG). Imaging was performed in the triggered mode (1 Hz) with an HP Sonos 5500 system (Hewlett Packard) equipped with a broadband transducer emitting at 1.8 MHz and receiving at 3.6 MHz. The tip of the transducer was carefully positioned between 4 and 5 cm from the epicardial surface, a distance corresponding to the focal distance of the transducer in the z axis.

Experimental Protocol
After initial isolation and surgical preparation, hearts were perfused at a constant flow rate (1.5 to 2 mL·min−1·g−1) and allowed to equilibrate for 30 minutes. LV end-diastolic pressure was initially set at 4 to 8 mm Hg by adjustment of the volume of the LV balloon. Once a stable level was attained, no further volume changes were made. At the end of the equilibration period and before the infusion of microbubbles was started, the hearts were exposed to triggered (1 Hz) ultrasound emissions for 5 minutes, with a mechanical index (MI) of 1.6. A solution of ultrasound contrast agents containing ∼8×1010 microbubbles·mL−1 was then infused at a constant rate of 1 mL/min. After 5 minutes of infusion, the hearts were again exposed to 5 consecutive triggered ultrasound sequences, each consisting of a 3-minute insonation period followed by a 2-minute recovery period.

The functional effects of acoustic pressure amplitude variations were evaluated in different groups of hearts. A first group of 6 hearts was exposed to ultrasound at an MI of 1.6 without any contrast agent. A second group of hearts (n=7) was exposed to ultrasound at increasing MIs from 0.6 to 1.6 in the presence of PESDA. The combined bioeffects of ultrasound and PESDA on myocardial function, coronary perfusion pressure, and microvascular integrity were further evaluated in 4 additional groups of hearts (n=6 each) in which the hearts were exposed to a single MI of 0.2, 0.6, 1.0, or 1.6. Finally, 3 groups of hearts were exposed to ultrasound at an MI of 1.6 in the presence of either Sonozoid, Optison, or Levovist.

Collection and Analysis of Samples
Coronary venous effluent was collected through the pulmonary artery, and PO2, PCO2, and pH were monitored regularly by means of a commercially available pH-blood gas analyzer. Glucose, troponin I, and lactate concentrations were determined in samples of aortic perfuse and pulmonary artery effluent at baseline and just before and at the end of the first ultrasound exposure, as well as just before and at the end of the last ultrasound exposure.

Morphological Analysis
After completion of each study, hearts were retrogradely perfused with erythrocyte-free Krebs-Henseleit buffer and fixed with a mixture of 3.5% formaldehyde-2.5% glutaraldehyde in phosphate buffer at pH 7.4. In some hearts, the fixation step was preceded by the perfusion of a PBS solution containing 100 IU/mL of the type VI peroxidase enzyme (Sigma). The hearts were then removed from the cannula and sliced into serial 1-mm-thick short-axis slices. One-cubic-millimeter samples of myocardium that was exposed (red zone) or not (white zone) to ultrasound were analyzed under the light microscope to quantify endothelial and capillary alterations. Morphometric assessment was performed on semithick sections stained with toluidine blue. Each section was examined to measure the percentage of capillaries and arterioles presenting with endothelial damage or rupture. A total of 1000 capillaries were examined for each zone of the sample. In hearts perfused with the peroxidase enzyme, the distribution of peroxidase activity was examined on semithick sections. For this purpose, sections were air-dried, washed with toluene and methanol (4 steps of 5 minutes each), and treated with PBS at pH 7.4. They were then incubated with 3-amino-9-ethylcarbazole (Dako A/S) for 10 minutes, rinsed with tap water, and mounted with Faramount (Dako A/S). Areas of peroxidase activity were quantified by planimetry.

Statistical Analysis
Values are mean±1 SD. Significance of differences was determined with an ANOVA for repeated measurements. Individual comparisons between groups were performed post hoc with the Bonferroni test. All tests were 2 sided, and a P value <0.05 was considered indicative of a statistically significant difference.

Results

Effects of Ultrasound and PESDA on LV Developed Pressure
Typical changes in LV developed pressure (LVDP) measured during ultrasound exposure in a heart perfused in the presence
or absence of PESDA are shown in Figure 1. As shown in Figure 2, ultrasound exposure in the presence of PESDA resulted in a progressive MI-dependent decrease in LVDP. The largest decrease in LVDP was found during exposure to an MI of 1.6. At this acoustic pressure, LVDP decreased from 86±12 to 71±12 mm Hg (83±5% of baseline). Figure 2 also shows the percent decrease in LVDP in hearts exposed to 5 consecutive ultrasound emissions at the same MI. Five groups of hearts were studied: control hearts exposed to an MI of 1.6 in the absence of any contrast agent and 4 groups of hearts receiving PESDA and exposed to MIs of 0.2, 0.6, 1.0, or 1.6. No significant decrease in LVDP was noted in hearts exposed to ultrasound alone or in those receiving PESDA and exposed to an MI ≤0.6. In the other 2 groups, a transient decrease in LVDP occurred during each exposure to ultrasound. The decrease in LVDP was reproducible between 2 ultrasound exposures and was greater at an MI of 1.6 than at an MI of 1.0. The maximal decrease in LVDP reached during exposure to an MI of 1.6 was 22±6% of baseline.

Effects of Ultrasound and PESDA on Coronary Perfusion Pressure

Figure 3 shows the percent changes in coronary perfusion pressure in hearts exposed to 5 consecutive ultrasound emissions at the same MI. As for LVDP, no significant changes in coronary perfusion pressure were observed in hearts exposed to ultrasound alone or in those receiving PESDA and exposed to an MI ≤0.6. In the other 2 groups of hearts, a transient but significant increase in coronary perfusion pressure was noted during each ultrasound exposure. The maximal increase in coronary perfusion pressure reached during exposure to an MI of 1.6 was 20±6%. Simultaneous measurements of myocardial lactate production indicated that the increase in coronary perfusion pressure during ultrasound exposure at MIs of 1.0 and 1.6 was always accompanied by significant release of lactate in the coronary effluent (Figure 3, bottom). There were no significant changes in the other biologic parameters measured.
Effects of Ultrasound and PESDA on Microvascular Integrity

Figure 4 shows the macroscopic appearance of a heart exposed to ultrasound alone and that of a heart exposed to PESDA and ultrasound at an MI of 1.6. Figure 5 shows the light microscopic appearance of myocardial regions located within or outside the beam elevation in 2 hearts exposed to both PESDA and ultrasound. In regions located outside the beam elevation, no morphological abnormalities were noted. By contrast, in the regions covered by the beam elevation, several capillary rupture sites with extravasation of red blood cells into the interstitial space could be identified. As shown in Figure 6, the percentage of ruptured capillaries increased with the intensity of ultrasound exposure and culminated at 3.6±1.4% at an MI of 1.6. In the hearts perfused with the peroxidase enzyme, peroxidase activity could never be found in the regions located outside the beam elevation or in regions exposed to MIs <0.6 (Figure 5B). By contrast, in the regions covered by the beam elevation and exposed to MIs ≥0.6, peroxidase activity was always present and covered 4.4±0.4% of the total muscle area in hearts exposed to an MI of 0.6, 14.1±3.4% in those exposed to an MI of 1.0, and 52.6±5.1% in those exposed to an MI of 1.6 (P<0.01, Figure 5D).

To rule out the possibility that the observed functional and morphological changes were due to aggregation or destruction of large microbubbles within the coronary arteries, additional experiments were conducted in which PESDA was filtered with a 5-μm filter. The decrease in LVDP in hearts exposed to an MI of 1.6 was similar whether filtered or unfiltered PESDA was used.

Influence of Machine Settings

The influence of the echographic system settings on the observed bioeffects was studied in additional experiments in which the pulse emission frequency or the pulse length was varied. Insonation at an emission frequency of 2.1 MHz induced a significantly lesser decrease in LVDP (from 91±6 to 81±3 mm Hg) than insonation at an emission frequency of 1.8 MHz (from 90±7 to 70±6 mm Hg, P<0.05 versus 2.1 MHz). By contrast, no significant differences were noted between hearts exposed to an MI of 1.6 and pulse lengths of 1, 2, or 4 cycles per pulse.

Role of the Contrast Agent

To test whether the observed bioeffects were specific to the contrast agent used, ie, PESDA, additional experiments were also conducted in which hearts were exposed to Sonozoid, Optison, or Levovist. In these hearts, the effects of ultrasound exposure on LVDP and capillary damage were similar to those observed with PESDA.

Discussion

Our data indicate that the interaction between ultrasound and microbubbles in the myocardium induces significant bioeffects, which consist of a transient and reversible decrease in LV contractile performance, a transient and reversible increase in coronary perfusion pressure, an increase in lactate production, and the occurrence of microvascular damage.
These effects appear to be independent of each other. Nonetheless, they appear to be related to the acoustic pressure amplitude and to require a threshold acoustic power to occur.

**Ultrasound-Induced Microvascular Damage**

Because of safety considerations, the possible interaction between ultrasound and tissue has received much attention. It is now well established that in organs containing air or in the presence of strong cavitation nuclei, such as contrast agents, ultrasound exposure may induce significant tissue damage, particularly to the microvasculature.8,9 There is also accumulating evidence that the mechanisms by which tissues are being damaged is the process of inertial cavitation. During this process, microbubbles experience extreme variations in their size, which may culminate in their physical destruction. It may also generate free radicals and cause microstreaming and shear stress secondary to the collapse and/or rapid translation movements of the microbubbles.8,10–14

Several investigators have reported on the occurrence of tissue hemorrhage and endothelial cell damage after ultrasound exposure of cultured cells and organs containing air, such as the lungs or the intestine. Dalecki et al4 reported extensive tissue exposure of cultured cells and organs containing air, such as the lungs or the intestine. Dalecki et al4 reported extensive tissue hemorrhage and endothelial cell damage after ultrasound microbubbles.8,10–14

and/or rapid translation movements of the microstreaming and shear stress secondary to the collapse and/or rapid translation movements of the microbubbles.8,10–14

Since the study of Skyba et al,2 we observed significant capillary damage in hearts exposed to both ultrasound and contrast agents, with no significant changes noted in hearts exposed to either ultrasound or contrast alone. In addition, our results indicate that at high MIs, the occurrence of capillary damage is independent of the contrast agent used. This is probably due to the fact that the 4 agents used in the present study are completely destroyed when exposed to MIs such as we used. However, we cannot rule out the possibility that differences could be observed at lower MIs, because initial bubble size and shell characteristics are known factors that influence the energy at which inertial cavitation and hence its bioeffects occur.

**Ultrasound-Induced Contractile Dysfunction**

Our data also indicate that the combined exposure of hearts to ultrasound and contrast agents may transiently alter contractile performance. Because this phenomenon was paralleled by an increase in coronary perfusion pressure and a rise in lactate production, it was likely related to the occurrence of transient myocardial ischemia. Much like capillary damage, contractile dysfunction was more pronounced when hearts were exposed to higher MIs. Nonetheless, given its transient nature, contractile dysfunction was probably unrelated to capillary damage.

The mechanisms by which ultrasound exposure induced transient ischemic dysfunction in the present study remain uncertain. Previous investigators have reported on the occurrence of reversible contractile dysfunction in dogs subjected to high-energy ultrasound delivered directly in the LV cavity by a high-power ultrasound generator.15 The phenomenon was accentuated under low loading conditions, in the presence of low plasma calcium concentration, and during mild selective \( \alpha_1 \)-adrenergic stimulation.16 The transient and reversible decrease in LV function during ultrasound exposure was attributed to ultrasound-induced endocardial endothelium dysfunction.17 Other investigators have shown that similar dysfunction could be obtained by selectively damaging the endothelial monolayer of the coronary bed.18 At variance with these earlier reports, in the present study, exposure of hearts to ultrasound alone, even at an MI of 1.6, did not produce any LV contractile dysfunction. This is probably because the energy levels used in the present study were considerably lower than those used in the previous reports. Nonetheless, we did observe significant dysfunction once contrast agents were added during ultrasound exposure. One possible explanation for these findings is that the addition of contrast agents made the endothelial cells more susceptible to the ultrasound energy, causing transient endothelial dysfunction and hence vasocostriction and ischemia. Alternatively, microbubble aggregation, which has also been shown to occur when microbubbles are exposed to ultrasound,3 may have provoked transient capillary and/or arteriolar plugging, which in turn caused ischemia and transient dysfunction.

**Implications for the Use of MCE in Humans**

The clinical relevance of our findings must be examined with caution. Although our data clearly demonstrate that significant microvascular damage and transient contractile dysfunction can be induced when hearts are simultaneously exposed to high-energy ultrasound and contrast agents ex vivo, the likelihood that such adverse events will ever occur in vivo, and hence in humans, is extremely small. First, the energy levels that caused capillary damage and transient contractile dysfunction in the present study are unlikely to be reached in vivo. Indeed, the threshold MI that caused these events in our experiments was 0.8. Because the ultrasound energy was...
administered directly to the heart, without any interposing medium and hence attenuation, it is probable that the energy that was delivered to these hearts was 30% to 40% higher than that which could be delivered in vivo, through the chest wall. Second, even if some damage could be provoked, it would most probably be quite localized and thus unlikely to induce any measurable functional effects. The human LV, which weighs between 150 and 180 g, is considerably larger than that of an isolated rabbit heart, which weighs between 5 and 7 g, 2 or 3 g of which was exposed to ultrasound and damaged (the red area on Figure 4). If such an amount (2 g) was to be damaged in a human heart, it would probably cause no functional harm. Only very subtle abnormalities, such as the occurrence of premature ventricular contractions, could potentially be seen. Thus, overall, the risk of adverse effects on the occurrence of ischemic injury in isolated perfused rabbit hearts. Circ Res. 1994;74:817–828.

It has also been suggested that ultrasound-induced capillary ruptures could be used as a means to enhance local tissue permeability and thereby facilitate the local delivery of drugs or genetic material. In the rat spinotrapezius muscle, Price et al recently demonstrated the feasibility of delivering colloidal particles through capillary ruptures caused by ultrasound-induced microbubble destruction. These data were confirmed very recently in rat hearts, in which the galactosidase gene was successfully delivered and expressed after their exposure to both ultrasound and contrast microbubbles. The present study confirms the ability of high-energy ultrasound in the presence of contrast microbubbles to increase capillary permeability and to facilitate the delivery of large macromolecules, such as the immunoperoxidase enzyme. Our study does show, however, that this can only be accomplished by using MIs that are unlikely to be reached during conventional diagnostic imaging. Transthoracic transducers emitting at a lower frequency, a higher MI, or both will probably be needed to achieve this goal. Intracardiac probes may be another alternative.

In conclusion, we have shown that exposure of isolated hearts to high-energy ultrasound and ultrasonic contrast agents provokes a transient but reversible contractile dysfunction, which is probably ischemic in origin, as well as limited capillary ruptures, which may provide the unique opportunity to increase tissue permeability and thereby facilitate local drug delivery.

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