Ultrasound Energy Improves Myocardial Perfusion in the Presence of Coronary Occlusion

Robert J. Siegel, MD, FACC,* Valentina N. Suchkova, MD,† Takashi Miyamoto, MD,* Huai Luo, MD,* Raymond B. Baggs, DVM, PhD,‡ Yoram Neuman, MD,* Michael Horzewski, BS,* Veijo Suorsa, PhD,* Sergio Kobal, MD,* Todd Thompson, BS,* Debra Echt, MD,* Charles W. Francis, MD†

Los Angeles, California; and Rochester, New York

OBJECTIVES We evaluated whether ultrasound improves myocardial tissue perfusion in 14 animals with coronary artery occlusion.

BACKGROUND A recent study demonstrated that low-frequency ultrasound improves tissue perfusion in the rabbit ischemic limb, but there are no data on ultrasound enhancement of myocardial perfusion.

METHODS Fourteen animals (9 dogs, 5 pigs) underwent thoracotomy and occlusion of a diagonal branch of the left anterior descending coronary artery. Myocardial tissue perfusion units (TPUs) and pH were measured before coronary occlusion, after occlusion, and after direct exposure of the ischemic myocardium in the presence of fixed occlusion to low-frequency ultrasound (27 kHz).

RESULTS The TPU decreased from 100.9 ± 13 at baseline to 71.1 ± 13 (p < 0.01) after 60 min occlusion but rose by 19.7% to 85.1 ± 8 (p < 0.01) after ultrasound exposure for 60 min. After 60-min coronary occlusion, myocardial pH fell from 7.43 ± 14 to 7.05 ± 0.15 (p < 0.01) but then improved to normal (7.46 ± 0.32) after ultrasound for 60 min. Administration of L-N-nitro-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, before ultrasound exposure, blocked improvement in myocardial tissue perfusion and pH by ultrasound. Quantitative histomorphology showed a significant increase in the capillary area of myocardium exposed to ultrasound versus non-exposed myocardium (16.2 ± 7.9 vs. 8.2 ± 2.1, p < 0.02).

CONCLUSIONS Low-frequency, low-intensity ultrasound improves myocardial tissue perfusion and pH in the presence of a fixed coronary artery occlusion. (J Am Coll Cardiol 2004;44:1454–8) © 2004 by the American College of Cardiology Foundation

The goal of reperfusion therapy is to re-establish tissue perfusion, whether using a noninvasive approach such as thrombolytic therapy or an invasive strategy of percutaneous coronary intervention. Investigators have pursued transcatheter ultrasound energy delivery for direct thrombus dissolution in vitro and in animal and human studies (1,2). Ultrasound has also been applied noninvasively to directly disrupt thrombi in animals (3) and to facilitate clot lysis with thrombolytic drugs in both animals and humans (4,5). Methods to improve myocardial tissue perfusion independent of reperfusion would also be desirable for the treatment of myocardial ischemia and infarction. Miyamoto et al. (6) found that noninvasive application of low-frequency ultrasound causes coronary arterial vasodilation. Recently, Suchkova et al. (7,8) demonstrated that low-frequency, low-intensity ultrasound improves tissue perfusion in ischemic rabbit limbs through a nitric oxide (NO)-dependent mechanism. Consequently, we evaluated whether low-frequency ultrasound improves myocardial tissue perfusion in the setting of a fixed coronary occlusion.

METHODS

Studies were performed according to the American Heart Association’s position on research animal use. Nine dogs and five pigs were anesthetized and maintained by isoflurane inhalation.

Ultrasound device. The ultrasound system consists of a generator and an ultrasound transducer delivering 27-kHz, pulsed-wave ultrasound (Timi3 Systems Inc., Santa Clara, California). Ultrasound, transmitted through a water-filled bladder, was applied directly to the myocardium at the beam maximum intensity of about 1.4 W/cm² (corresponding to spatial-average, temporal-average intensity [ISATA] of about 0.13 W/cm²) and a pulsed-wave duty cycle of 30%. The water-filled bladder was coupled to the myocardium with ultrasound gel. The shape of the ultrasound field diverges and covers the entire heart. A calibrated hydrophone (model 8103, Bruel & Kjaer, Naerum, Denmark) was used to measure the output of the transducer. The ultrasound field has previously been described elsewhere (6).

Experimental protocol (Fig. 1). Thoracotomy was performed to expose the heart. Baseline measurements of
myocardial perfusion were obtained with a laser Doppler flow meter (BFL21, Transonic Systems, Ithaca, New York) with tissue perfusion units (TPUs) that are linearly related to the red cell number multiplied by their velocity in the measurement volume, as described previously (9). The light penetrates the muscle about 1 mm and the surface area measured is 1 mm². A pH meter (model HJ9023C, Hanna Systems, Woonsocket, Rhode Island) was used to measure myocardial pH (9).

After baseline measurements of myocardial TPU and pH, the diagonal branch of the left anterior descending coronary artery perfusing that area of the heart was occluded with vascular loops. Subsequently, the TPU, tissue pH, and myocardial temperature beneath the ultrasound transducer were measured at 30 and 60 min.

After 60-min coronary occlusion, low-frequency pulsed ultrasound (27 kHz, 30% duty cycle) was applied to the myocardium subtended by the occluded coronary artery for 1 h. Ultrasound exposure was interrupted every 30 min to measure TPU, pH, and temperature. In three dogs, L-N-nitro-arginine methyl ester (L-NAME) (50 mg/kg), an inhibitor of NO synthase (NOS), was given intravenously 10 min before ultrasound exposure. The TPU and pH were also measured 30 min after completion of ultrasound exposure. Subsequently, the coronary ligation was reversed, and 15 min later, TPU and pH were measured.

Two animals did not receive ultrasound and served as controls, one of which received nitroglycerin. After coronary occlusion, the nitroglycerin was titrated to reduce the systolic arterial pressure by 10 to 20 mm Hg.

**Histopathology.** Animals were euthanized, and the myocardium was fixed in 10% formalin. In each canine heart, representative tissue blocks were taken from multiple sites unexposed to ultrasound and exposed to ultrasound both proximal and distal to the site of coronary ligation. Tissues were sectioned at 4 μ, stained with hematoxylin-eosin, and examined by an investigator (Dr. Baggs), who was blinded as to the site and pretreatment. Transverse histologic sections of muscle tissue were examined using an Olympus AH-2 photomicroscope with a SPOT high-resolution camera coupled to Image-Pro Plus software. Calibration utilized an American Optical stage micrometer. Using the tracing tool, the circumference of 10 capillaries was measured, sectioned at 5 μ, and stained with hematoxylin-eosin.

**Statistical analysis.** All data are presented as the mean value ± SD. Overall comparisons of the time course of changes in TPU, pH, and temperature were performed by repeated measures analysis of variance (ANOVA). If the overall ANOVA model was significant, then paired t tests were used to assess the change between specific time points (baseline to 30 min of occlusion, baseline to 60 min of occlusion, 60 min of occlusion to 30 min of ultrasound, and 60 min of occlusion to 60 min of ultrasound). Change in capillary area was also assessed by the paired t test. A significance level of 0.05 was used for all tests. No adjustment for multiple comparisons was made.

**RESULTS**

Figure 2A demonstrates the tissue perfusion data on eight dogs and four pigs at baseline, 30 and 60 min after coronary artery ligation, 30 and 60 min after ultrasound exposure, 30 min after stopping ultrasound, and 15 min after re-establishing coronary flow by releasing the vascular loop. The baseline TPU of 104.8 ± 14 fell to 79.4 ± 13 after 30 min and to 73.1 ± 13 (p < 0.05) after 60-min occlusion. After application of low-intensity, 27-kHz ultrasound, tissue perfusion increased by 18.9%, from 73.1 ± 13 to 86.9 ± 14 (p < 0.05). Thirty minutes after termination of ultrasound exposure, TPU again declined to pre-ultrasound values of 73.4 ± 9. Restoration of coronary flow by release of the vascular loop resulted in an increase in TPU to 97 ± 11. As shown in Figure 2B, the myocardial pH fell from a baseline value of 7.44 ± 0.13 to 7.20 ± 0.08 after 30 min and to 7.06 ± 0.21 after 60-min coronary occlusion (p < 0.05). As with TPU, the pH rose after application of ultrasound to 7.36 ± 0.26 (p < 0.05) after 30 min and after 60 min of ultrasound to 7.44 ± 0.30 (p < 0.05). Unlike TPU, however, 30 min after stopping ultrasound, the pH remained elevated above the pre-ultrasound level (7.28 ± 0.29 vs. 7.06 ± 0.21), but less than the 60-min ultrasound value (7.28 ± 0.29 vs. 7.44 ± 0.30). After opening the

![Figure 1](image-url)

**Abbreviations and Acronyms**

- L-NAME = N-Nω-nitro-arginine methyl ester
- NO = nitric oxide
- NOS = nitric oxide synthase
- TPU = tissue perfusion unit
ligation, the pH value (7.44 ± 0.12) was similar to that at baseline and during ultrasound exposure.

In Figures 2C and 2D, data on each species (dogs and pigs) are plotted separately for myocardial TPU and pH. The TPU and pH behaved similarly in dogs and pigs after coronary occlusion, ultrasound exposure, and after release of coronary ligation. Ultrasound induced a significant increase in perfusion and pH in both species (p < 0.05).

Three dogs were pretreated with L-NAME 10 min before ultrasound exposure to inhibit NOS. As demonstrated in Figures 2A and 2B, the application of ultrasound to ischemic myocardium in these animals resulted in no improvement in tissue perfusion, as measured by TPU (46.8 ± 13 vs. 44.9 ± 5) or pH (7.10 ± 0.03 vs. 7.07 ± 0.03). In addition, 30 min after reversal of coronary occlusion by removal of the ligature, the baseline myocardial tissue perfusion (TPU: 46.8 ± 13 vs. 55.2 ± 13) and pH (7.10 ± 0.03 vs. 7.16 ± 0.06) were not re-established.

Two animals served as controls and received no ultrasound exposure. After coronary occlusion, there was no spontaneous improvement in perfusion or pH. In one dog receiving intravenous nitroglycerin, tissue perfusion and pH did not improve after 30, 60, or 90 min.

**DISCUSSION**

This is the first study demonstrating that low-frequency, low-intensity ultrasound improves myocardial tissue perfusion in the presence of a fixed coronary artery occlusion. Previous work has shown that low-frequency ultrasound

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*Figure 2.* The myocardial tissue perfusion unit (TPU) (A and C) and pH (B and D) are shown at baseline, 30 and 60 min after coronary occlusion, 30 and 60 min of ultrasound exposure, 30 min after stopping ultrasound, and 15 min after opening the coronary artery. Panels A and B are combined data from eight dogs and four pigs. The hashed lines demonstrate the results when the animal (three dogs) was pretreated with L-NAME. C and D show the data for each species (dogs and pigs). The thin line indicates dog data, and the thick line is for pig data. *p < 0.05 for 60-min occlusion versus during ultrasound at 30 or 60 min and after opening the coronary artery.
energy applied transthoracically has a significant coronary vasodilator effect in dogs (6). As in the rabbit peripheral artery studies of Suchkova et al. (7,8), we found that application of ultrasound in both a canine and porcine model resulted in enhanced myocardial tissue perfusion, as measured by both laser Doppler capillary flow and tissue pH, despite persistent coronary artery occlusion. Because dogs have a more robust collateral circulation than do humans, we thought it was important to also corroborate our findings in pigs, which, like humans, lack an innate collateral circulation (10). Of note, after ultrasound, the TPU rose to 85% of their baseline value, and the pH normalized to 7.40, a level at which cells are likely to be viable and functional. Ultrasound-enhanced perfusion in the setting of epicardial coronary artery occlusion was associated with histologic evidence of an increase in capillary circumference or dilation. As in the previous study on peripheral muscle (7), this effect was related to the synthesis of NO because an inhibitor of NOS blocked the ultrasound increase in tissue perfusion, improvement in pH, and increase in myocardial capillary size.

The mechanism(s) by which low-frequency ultrasound improves tissue perfusion is complex. Low-frequency ultrasound dilates epicardial coronary arteries as well as peripheral arteries (11–13). Ultrasound generates pressure waves, which impart mechanical energy to the tissues (7). The observed non-thermal vasodilatation by ultrasound is likely to be due to a direct vasodilator effect related to oscillation or turbulent shear stress. Alterations of the endothelial cell mechanoreceptors may thus promote the release of NO (14). The primary role of NO for enhancing tissue perfusion, despite epicardial coronary occlusion, is supported by the blocking effect of L-NAME. This finding is consistent with studies of peripheral limbs in which inhibition of NOS also eliminated any ultrasound augmentation of muscle perfusion (7,8). A range of L-NAME concentrations have been used to successfully block NOS. In this study, we used a similar dose of L-NAME, as used in previous reports. L-NAME, at the concentration used (50 mg/kg), blocks ultrasound-induced upregulation of NOS (7,8,15–20). Therefore, ultrasound-enhanced myocardial tissue perfusion in the setting of coronary occlusion is likely to be due to NO-mediated enhancement of coronary artery collateral flow. Tissue perfusion, as measured in TPU, decreased to 70% of baseline after arterial occlusion but rose to 85% of pre-occlusion levels during ultrasound exposure. This finding, together with the significant increase in tissue pH and increase in capillary area after ultrasound, are consistent with an increase in collateral flow to the ischemic zone.

Multiple studies have demonstrated that ultrasound has a vasodilator effect. Catheter-delivered low-frequency ultrasound has been shown to induce arterial vasodilation ex vivo in the rabbit aorta, in canine coronary arteries, and also in a clinical study in which patients were treated with peripheral arterial ultrasound angioplasty as well as in patients with peripheral arterial vasospasm (12,13). Two previous studies in rabbit limbs have demonstrated ultrasound-enhanced perfusion in the setting of complete arterial occlusion (7,8), via an NO-dependent mechanism. Our findings are consistent with previous studies but extend these observations to the critical setting of acute myocardial ischemia from epicardial coronary artery occlusion.

Transcatheter ultrasound energy has been used effectively to directly disrupt thrombi in vitro and in vivo in animals and in humans (1,2,20,22). More recently, the noninvasive application of ultrasound has been developed for thrombus removal. Rosenschein et al. (3) have successfully applied high-intensity, focused ultrasound for site-specific thrombus destruction in vivo and in a porcine model. We have shown that low-frequency, low-intensity ultrasound speeds the rate and efficacy of thrombolytic drugs in vitro and in animal studies (4,8,20,23–26). A pilot study has corroborated the safety and feasibility of using low-frequency...
ultrasound to augment coronary thrombolysis in patients with acute myocardial infarction (5). The findings of the current study indicate that ultrasound energy alone can improve tissue perfusion and reverse acidosis. Consequently, ultrasound therapy has substantial promise as therapy for patients with acute coronary syndromes (with or without a thrombolytic drug) and for the treatment of chronic myocardial ischemia.

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Reprint requests and correspondence: Dr. Robert J. Siegel, Division of Cardiology, Room #5335, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, California 90048. E-mail: siegel@csbs.org.

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