Myocardial Lesion Formation Using High-intensity Focused Ultrasound

David J. Engel, MD, Robert Muratore, PhD, Kumiko Hirata, MD, Ryo Otsuka, MD, Kana Fujikura, MD, Kenichi Sugioka, MD, Charles Marboe, MD, Frederic L. Lizzi, EngScD, and Shunichi Homma, MD, *New York, New York*

Background: **The potential therapeutic uses of ultrasound energy in cardiac disease have not been extensively studied. We have developed a means to deliver high-intensity focused ultrasound (HIFU) to myocardial tissue. Unlike other therapy modalities such as radiofrequecy catheter ablation, this system has the advantages of not requiring direct tissue contact and the ability to focus intense energy within a small volume.**

Methods: **Sections of left and right ventricles from freshly excised canine hearts were treated in vitro with HIFU pulses. Lesions were created using 1-second HIFU pulses with ultrasonic powers ranging from 19.8 to 45.8 W.**

Results: **There was a dose-response relationship between the applied HIFU energy and lesion size (r = 0.70, P < .001). Myocardial lesion formation with HIFU was also performed in vivo in a canine open-chest beating heart model. With 200-millisecond HIFU pulses gated to the electrocardiogram, focal myocardial lesions were created ranging in length from 2 to 6 mm depending on the dose used. Furthermore, both in vitro and in vivo, focal lesions were successfully formed in the midmyocardial wall that spared both the endocardial and epicardial surfaces.**

Conclusion: **HIFU is a novel means to create focal myocardial lesions without direct tissue contact. HIFU energy delivery can be gated to the electrocardiogram in an in vivo model, and lesions can be formed intramyocardially. Further application of this technology may prove to be useful for the ablation of myocardial lesions such as arrhythmogenic foci and the hypertrophic ventricular septum in hypertrophic cardiomyopathy.**

The potential therapeutic uses of ultrasound energy in cardiac diseases have not been well studied. We tested a novel system to deliver high-intensity focused ultrasound energy in vitro and in vivo to canine myocardial samples without direct contact with the target tissue. Focal myocardial lesions were formed in a dose-dependent manner, and myocardial lesions were created. This technology may prove useful for ablation of focal intramyocardial lesions such as arrhythmogenic foci and the hypertrophic left ventricular septum in hypertrophic cardiomyopathy. (J Am Soc Echocardiogr 2006;19:932-937.)

High-intensity focused ultrasound (HIFU) is capable of producing focal lesions within various human tissues and has achieved clinical success in the treatment of disorders such as glaucoma and prostate cancer.[1,2] However, the potential therapeutic uses of ultrasound energy in cardiac disease have not been extensively studied. As a method to create focal lesions, HIFU offers several potential advantages over other therapy modalities. Unlike microwaves, it can be readily focused within small volumes.[2,3] It does not have the cumulative risks associated with ionizing radiation, and it is unaffected by the optical opacities that block laser penetration.[3,4] Furthermore, in contrast to radiofrequency (RF) waves, HIFU does not require direct contact with target tissue.

The use of HIFU to create focal, ablative lesions in the heart has been reported in experimental models,[5-7] but the experience has been limited in comparison with the widespread use of RF current. Previous studies using HIFU in the heart have been limited by the length of required exposure time, approximately 10 to 60 seconds needed to form myocardial lesions.[5-7] Given cardiac motion and cooling from blood flow, exposure times of this length are impractical for in vivo implementation. As such, we have designed a HIFU system capable of delivering significantly higher energy than has previously been achieved. The purpose of this study

From the Division of Cardiology, Columbia Presbyterian Medical Center, and Riverside Research Institute (R.M., F.L.L.).

Supported in part by: (1) Bioengineering Research Partnerships grant 5RO1 CA84588 from the National Cancer Institute and the National Heart, Lung, and Blood Institute (NHLBI); and (2) Cardiovascular Fellowship Training grant T32HL07854 from NHLBI.

Reprint requests: Shunichi Homma, MD, Columbia Presbyterian Medical Center, 622 W 168th St, PH 3-342, New York, NY 10032 (E-mail: *sh23@columbia.edu*).

0894-7317/$32.00

doi:10.1016/j.echo.2006.02.012

was to assess the feasibility and characteristics of myocardial lesion formation using this system.

METHODS

In Vitro Experimental Preparation

Ten adult mongrel dogs weighing 25 to 30 kg were used. Anesthesia was induced with 5 to 7 mg/kg of intravenously administered thiopental and maintained on inhaled 1.5% to 2.0% isoflurane during mechanical ventilation. A sternotomy was performed and the pericardial sac was opened to expose the heart. Cardiac arrest was induced with an overdose of 120 mg/kg of intravenously administered pentobarbital. In 8 cases the heart was excised, sectioned, and placed in a 37°C saline bath. For the two whole heart in vitro experiments, the excised hearts were left intact in the 37°C saline bath. The animals were cared for in accordance with the Guiding Principles for the Use and Care of Laboratory Animals (National Institutes of Health publication 82-23, 1985).

HIFU Delivery System

Therapeutic ultrasonic energy was supplied with an ultrasound therapy device (model CST-100, Sonocare).[3] The therapy transducer was a PZT-4 spherical cap transducer (80-mm diameter, 90-mm focal length) with a central 23-mm hole through which a diagnostic transducer (model MD3657, Panametrics, Waltham, Mass) was inserted and aligned to be coaxial and confocal with the therapy transducer. The diagnostic transducer had a 7.5-MHz nominal central frequency, 15-mm diameter, and 60-mm focal length; it contained a 3-mm central hole that housed a fiber-optic channel projecting a light alignment beam (Figure 1). The therapy transducer was excited through a tuned matching network to its third harmonic, 4.67 MHz. The HIFU energy was manipulated in both an intensity-dependent and time-dependent manner; nominal acoustic power varied from 19.8 to 45.8 W, and therapy pulse duration was set between 200 milliseconds and 1 second. The half-power beam width of the therapy beam was measured as 0.35 mm using a pulse-echo reciprocity technique, and ultrasonic power was measured using a radiation force technique with an absorptive target.[4]

The transducer was housed in an acrylic resin cone with a 25-mm diameter exit hole. The cone was filled with filtered degassed water, and the exit hole was covered with a latex membrane. Sections of left ventricle (LV) and right ventricle of the freshly excised canine hearts were mounted on viscoelastic rubber sheets (Sorbothane, McMaster-Carr, New Brunswick, NJ) and placed in degassed normal phosphate-buffered saline. The tip of the cone was immersed in the degassed phosphate-buffered saline bath that held the cardiac tissue sample. The position of the transducer was adjusted so that its focal point was at or 0.5 cm below the epicardial surface of the myocardial samples so that lesions could be created at different depths in the myocardium. Fifty myocardial lesions were created on ventricular sections from 8 hearts using HIFU pulses of varying intensities fixed at 1-second duration. For the whole heart in vitro experiments, 23 lesions were created in the LV free wall in two hearts, also at two different depths. Each lesion was formed with between 10 and 25 therapy pulses of 200-millisecond duration at a nominal acoustic power of 45.8 W.

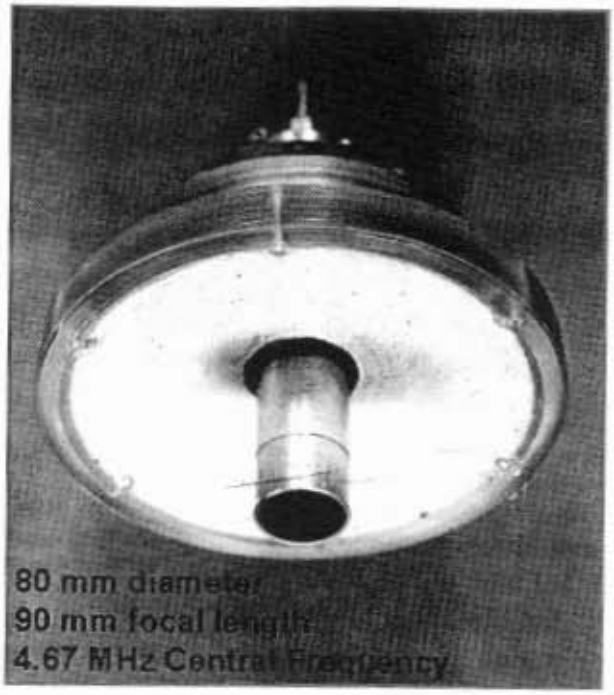

Figure 1 High-intensity focused ultrasound transducer.

In Vivo Lesion Formation

Two dogs were anesthetized and prepared as noted above. A left lateral thoracotomy and a pericardiotomy were performed to expose the LV free wall. The therapy transducer was placed in a cone filled with degassed phosphate-buffered saline over the epicardial surface of the beating LV free wall such that the focal point was set at or 0.5 cm below the epicardial surface. Trigger for the HIFU pulses was the R wave of the electrocardiogram (ECG) such that each pulse was delivered at the same point in the cardiac cycle (end diastole). Pulses of short duration, 200 milliseconds, were used to minimize the effect of cardiac movement on location of the treated area. Fourteen separate HIFU lesions were formed in each dog with 10 to 25 pulses at a nominal acoustic power of 45.8 W. Pulses were delivered on every fourth R wave of ECG to minimize the chance of inducing arrhythmias.

Gross and Histopathologic Evaluation

Myocardial sections containing HIFU-treated lesions were fixed in 10% buffered formalin immediately after lesion formation. Grossly visible lesions were sectioned and the maximum length of the lesion recorded. The tissue blocks

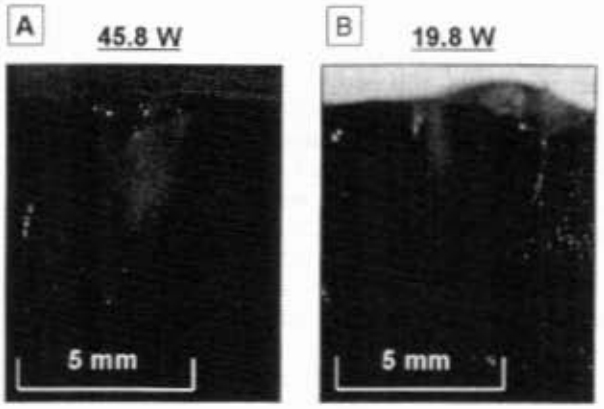

Figure 2 Representative high-intensity focused ultrasound–treated myocardial lesions using power of 45.8 W (**A**) and 19.8 W (**B**) with 1-second pulses.

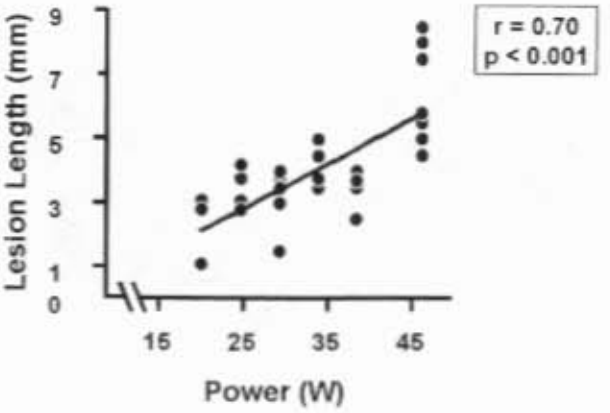

Figure 3 Dose-response relationship between high-intensity focused ultrasound energy and lesion size.

containing the lesions were embedded in paraffin, and myocardial sections that were 5-μm thick were fixed onto glass slides and stained with Masson trichrome stain.

Transmission Electron Microscopy

Myocardial sections were fixed in 3% glutaraldehyde and postfixed in 1% osmium tetroxide. These sections were then dehydrated in graded alcohol washes and embedded in Lx-112 (Ladd Research Industries, Williston, Vt). Semithin (1-μm) sections were stained with Toluidine blue O, and thin sections (60 nm) were stained with uranyl acetate and lead citrate. They were viewed by transmission electron microscopy (JEOL EXII-12000, JEOL USA, Inc., Peabody, Mass).

Statistical Analysis

A linear regression analysis was performed to examine the relationship between HIFU power and lesion length.

RESULTS

Gross and Microscopic Pathology

Figure 2 shows representative examples of the focal myocardial lesions formed in vitro. As shown, the gross lesions were clearly visible with distinct borders. Figure 2, *A*, demonstrates a lesion of 5.5 mm in length created in an LV section with a HIFU power of 45.8 W. Figure 2, *B*, demonstrates a 2.8-mm lesion created in an LV section with a HIFU power of 19.8 W.

Dose–Response Relationship

Figure 3 shows the group data for lesion length from a set of lesions (n = 31) created in LV samples using HIFU of varying intensities. The duration of HIFU delivery for all lesions was 1 continuous second, and the distance between the transducer and the surface of the tissue was fixed at 9.0 cm. As shown, the length of the myocardial lesions increased in a linear fashion with the power of the HIFU pulse ($r = 0.70$, $P < .001$).

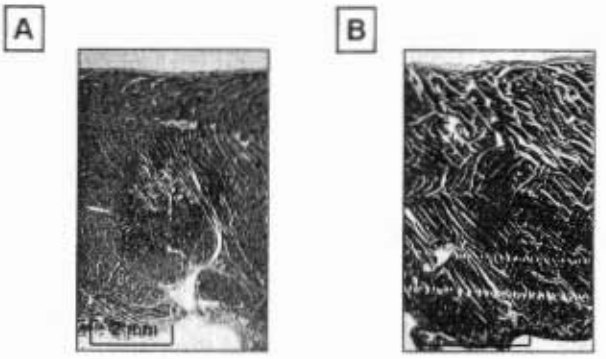

Figure 4 Representative high-intensity focused ultrasound–treated intramyocardial lesions using power of 45.8 W (**A**) and 29.0 W (**B**) with 1-second pulses.

Intramyocardial Lesion Formation

Figure 4 shows representative example of myocardial lesions formed with the HIFU transducer 8.5 cm from the surface of the tissue, thus, with the focal point 0.5 cm below the epicardial surface. As shown, distinct and focal lesions were formed that spared both the endocardial and epicardial surfaces of the tissue.

Histopathology

Figure 5 shows representative examples of myocardial sections stained with Masson trichrome and demonstrates the pathologic features of the lesions created with HIFU. As shown in Figure 5, *A*, the HIFU lesion appeared hypereosinophilic as a result of clumping of cytoplasmic materials. This pattern of injury is consistent with protein denaturation caused by thermal injury.[8] As demonstrated in Fig-

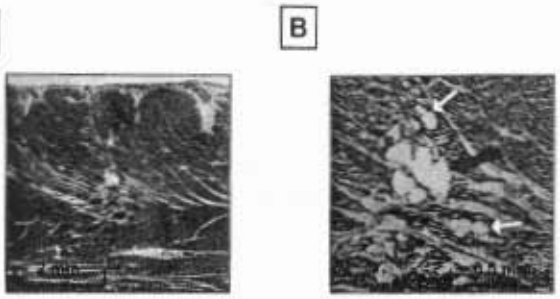

Figure 5 Histopathology of high-intensity focused ultrasound–treated myocardial lesions. (Original magnifications: **A**, ×10; **B**, ×20.)

ure 5, *B*, vacuoles were seen within individual myocytes and between myocytes in the interstitial space. These larger vacuoles in the interstitium appeared to disrupt the normal cellular architecture of the myocardium.

Transmission Electron Microscopy

Figure 6, *A* and *B*, shows the electron micrographs of a myocardial section in the control and HIFU-treated sections. Figure 6, *A*, demonstrates the typical ultrastructural features of a normal myocyte with the normal organization of myofilaments, contractile elements (*Z* bands), and mitochondria. Figure 6, *B*, shows the disruption of the normal intracellular architecture created by HIFU with scattered and fragmented mitochondria, and clumping and fragmentation of the myocyte Z bands. Figure 6, *C*, shows a separate HIFU-treated myocardial section that demonstrates the vacuolization both within the myocyte bordered by the sarcolemma and the large area of vacuolization in the interstitial space between the individual myocytes.

In Vivo Myocardial Lesion Formation

In the whole heart in vitro experiments, distinct myocardial lesions of varying sizes ranging from 6 to 12 mm in length were created using repetitive HIFU pulses (range 10-25), each of 200-millisecond duration every 4 seconds, at a power of 45.8 W. As such, similar energy delivery algorithm was adapted for in vivo experiments. There was no hemodynamic compromise or induction of sustained arrhythmias during lesion formation although isolated premature ventricular contractions frequently occurred immediately after HIFU pulse delivery. Figure 7 shows representative examples of the focal intramyocardial lesions that were created in vivo. As with in vitro experiments, distinct and focal myocardial lesions were readily created. The size of the visible HIFU lesions was smaller than in whole heart in vitro experiment, and ranged from 2 to 6 mm in length.

DISCUSSION

The results of this study demonstrate that focal myocardial lesions can be formed in vitro and in vivo with intense ultrasound energy without ultrasound transducer being in contact with the target tissue. Lesion size could also be readily manipulated in a dose-dependent manner. In addition, well-defined intramyocardial lesions could be formed without affecting endocardial or epicardial surfaces of the myocardium. The histopathology of the HIFU-treated lesions confirmed the ability of HIFU to create definitive cellular destruction as indicated by electron microscopic findings.

Ultrasound energy offers a potential alternative to current methods such as RF ablation to create therapeutic and focal myocardial lesions. The features of the pathologic injury to the myocardial tissue are comparable between RF ablation and HIFU therapy, which suggests that HIFU can potentially achieve similar clinical efficacy as RF ablation.[9,10] A pathologic feature of the HIFU-treated lesions not reported in RF induced lesions is the appearance of vacuolization both within and between individual myocytes. HIFU energy can be concentrated in a small cylindric volume in the order of 1 mm × 1 cm. Thus, a great deal of heat is generated within a small and precise volume that results in vaporization of intracellular water and vacuole formation. RF current, on the other hand, is less precisely focused as the generated heat decreases proportionally from the distance of the catheter delivering the current.[11]

Another potential advantage HIFU offers over RF ablation is that a catheter does not need to make direct contact with the target tissue. This may provide a means to reach potential therapeutic targets in the heart not directly accessible with a catheter such as layers of the myocardium other than the endocardium where specific targets such as arrhythmogenic foci may be located. The HIFU system is supposed to be suitable for a therapy of arrhythmia. Because trigger for the HIFU pulses was the R wave of the ECG, ultrasound energy could be delivered to the same cardiac spots in patients with arrhythmia such as atrial fibrillation and flutter. The noninvasive and focused nature of this therapeutic modality may also be useful to ablate hypertrophic ventricular septum in hypertrophic cardiomyopathy, a disorder in which an invasive ablative method such as cardiac surgery or intracoronary ethanol injection are currently used.[12,13]

The results of this study help to further advance the known experience with therapeutic ultrasound in the heart. The HIFU system reported in this study differs greatly from those in previous studies in that the focal-point intensities we generated were up to 10 times higher than those previously described.[5-7] This ability to increase power while maintaining a

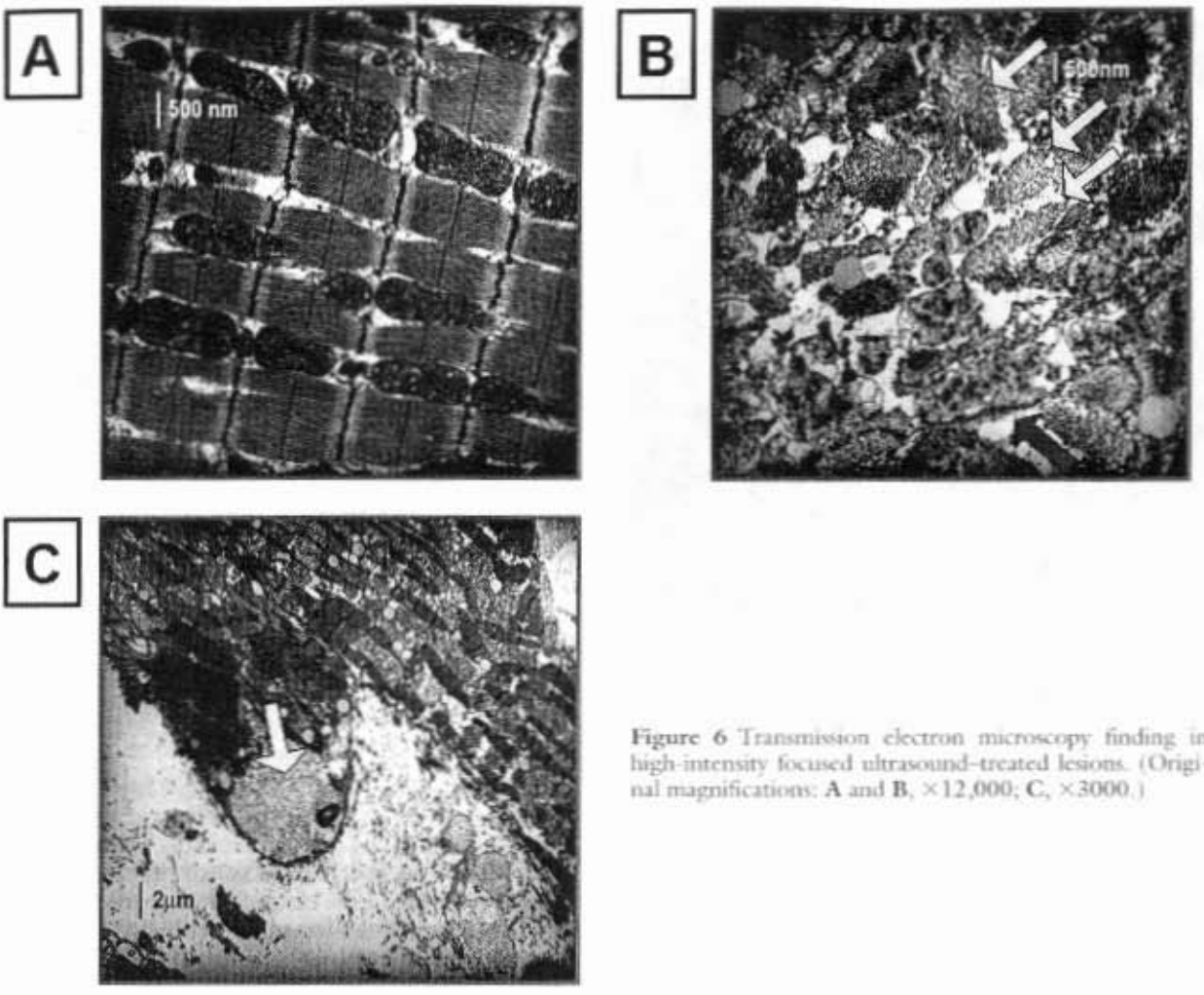

Figure 6 Transmission electron microscopy finding in high-intensity focused ultrasound–treated lesions. (Original magnifications: **A** and **B**, ×12,000; **C**, ×3000.)

distinct focal point helps to explain how we were able to generate distinct focal lesions with far fewer and shorter HIFU applications than has ever been reported.[5-7] This has been made possible by the use of large diameter transducers that were strongly focused, with f-numbers near one. Of note, the size of in vivo lesions were smaller than those achieved in vitro. We believe this was most likely because of loss of heat at the target area from circulating blood. In addition, because the heart moves in vivo during the 200-millisecond pulse, energy delivered was distributed to a larger area than if the target was stationary, resulting in a lower concentration of delivered energy.

Previous in vitro studies using HIFU in ventricular myocardium demonstrated that visible lesion formation required at least 15 seconds of HIFU application, as opposed to 1 second used in our study.[5] In a similar fashion, the HIFU system used in our study dramatically decreased the time needed to form a visible lesion in vivo in an open-chest beating heart model. In a prior study in which HIFU pulses were delivered to create AV block, lesion formation required an exposure time of 120 seconds.[6] In the current study, similarly sized in vivo lesions were formed with HIFU pulses, gated to the ECG, in 2 to 5 seconds. Given the challenges of creating focal lesions in a moving target, this ability to form myocardial lesions rapidly represents a very important advance with potential clinical applications. Furthermore, the future HIFU application in closed-chest models will require high power to assure delivery of adequate energy through intervening tissue.

The immediate challenge for upcoming experiments will be to combine a diagnostic ultrasound transducer with the therapy transducer to allow for more targeted focusing of the HIFU energy and real-time assessments of lesion formation. Real-time assessments are important because of the natural variations in tissue structure, suggested by the scatter in Figure 4. As our understanding expands on HIFU, therapeutic cardiac ultrasound system will become a reality in the clinical practice of cardiology as is the case in other medical specialties.

Figure 7 Two focal in vivo intramyocardial lesions (*arrows*) using power of 45.8 W with electrocardiographically gated 200-millisecond pulses.

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