Extracardiac Ablation of the Left Ventricular Septum in Beating Canine Hearts Using High-Intensity Focused Ultrasound

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Background: High-intensity focused ultrasound (HIFU) produces immediate focal lesions without direct tissue contact. Previously, we reported the HIFU potential for cardiac ablation. The purpose of this study was to evaluate the possibility of myocardial ablation in the left ventricle of beating dog hearts with monitoring by 2-dimensional echocardiography.

Methods: The operating frequency and the acoustic intensity were 5.25 MHz and 23 kW/cm², and the focal length and diameter were 3.3 mm axial and 0.37 mm wide at a distance of 35 mm from the transducer. Three dogs underwent a left-sided thoracotomy. The right ventricular surface was coupled with the transducer. The timing of the HIFU exposure was set during the early systolic phase using an electrocardiographic triggering system. The focal point was set in the left ventricular septum using 2-dimensional echocardiography mounted in the HIFU transducer. Ultrasound energy was delivered for 0.2 seconds. For each dog, we created 18 lesions. Exposures were performed 20, 30, or 40 times. Lesion size was assessed by manually measuring its length and width.

Results: All lesions except one were clearly visible. The histologic lesion area was $18.7 \pm 8.3$, $26.3 \pm 8.7$, and $35.5 \pm 15.7$ mm² (20, 30, and 40 times, respectively). The intraclass correlation coefficients were found to be 0.72, 0.63, 0.75, and 0.73 for lesion length, width, area, and depth, respectively.

Conclusion: HIFU can be used to create targeted, well-demarcated thermal lesions in the ventricular septum myocardium during cardiac contraction. (J Am Soc Echocardiogr 2007;20:1400-1406.)

High-intensity focused ultrasound (HIFU) is a non-invasive extracorporeal therapeutic technique with a possibility of thermally ablating structures without injuring intervening tissues.¹ Ultrasonic energy can be applied in a target volume to induce molecular agitation, absorptive heating, and thermal coagulative tissue necrosis.²⁻⁴ Several studies have examined the histologic changes related to HIFU ablations in the liver, kidney, prostate, breast, and brain.¹⁻⁵⁻¹² HIFU is being explored as a therapeutic modality in almost every tissue that is accessible by ultrasound. However, there are few studies that evaluate HIFU ablation for cardiac tissues.

Previously, we reported a novel system to deliver HIFU energy in vitro and in vivo to canine myocardial samples without direct contact with the target tissue.¹² HIFU offers several potential advantages over other therapy modalities as a technique to create focal lesions because HIFU does not apply ionizing radiation and does not require direct contact with target tissues.

We modified our previous transducer to a smaller one in which a 2-dimensional transducer can be mounted, and developed our software of HIFU exposure. We hypothesized that HIFU would have a potential to ablate the myocardium by thermal ablation and the new transducer could visualize the ablation. The purpose of this study was to assess the feasibility of HIFU ablation in the left ventricular (LV) basal septum of beating canine hearts and the possibility of visualization of HIFU ablation.

METHODS

HIFU System Combined with Diagnostic Echocardiography

The HIFU system consists of a signal generator (33250A, Agilent, Palo Alto, Calif), a power amplifier (ENI 2100L, Spectra Test Equipment Inc, Mountain View, Calif), and
33-mm spherically curved HIFU transducer made from piezoelectric ceramic. We used a commercially available digital ultrasound system (Sequoia 512, Acuson, Mountain View, Calif) for monitoring the focal site. The HIFU transducer’s focal length was 35 mm. It had a central hole (diameter 12.5 mm) that housed a 7.0-MHz B-mode diagnostic transducer (7V3C probe, Acuson). The diagnostic transducer was aligned to be coaxial and confocal with the HIFU transducer. The operating frequency of the HIFU transducer was 5.25 MHz and the ultrasound energy was applied with an acoustic intensity of 23 kW/cm². Acoustic intensity was measured with a microscale and perfect absorber. The focal zone beam shape was measured using a pulse echocardiographic technique with a point target at the half-power points; the focal zone was 3.3 mm in depth and 0.37 mm wide (Figure 1, A). The focus of the ultrasound beam was positioned at the desired tissue location by distance measurements made with the diagnostic B-mode transducer.

**HIFU Ablation In Vivo Study**

Three mongrel dogs of either sex (22 kg; range 20-26 kg) were used for this study. Anesthesia was induced with thiopental (15-17 mg/kg) intravenously. Each dog was intubated and maintained in a state of deep anesthesia.
using inhaled isoflurane. The surface electrocardiography was displayed and recorded on a multichannel recorder (RS3800, Gould Inc T and M, Valley View, Ohio). A right-sided thoracotomy was performed to place the ultrasound probe, covered in a sterile sleeve, over the heart. We located the right ventricular anterior wall and put the sterile echocardiographic jelly and plastic bath filled with degassed water on the dog heart. The transducer was placed in the bath and positioned over the canine heart (Figure 1, B). The right ventricular surface was coupled to the transducer using echocardiographic transmission gel and a degassed water bath. The focus of the HIFU transducer was targeted at the center of the basal LV septum wall using the diagnostic 2-dimensional transducer mounted in the center of HIFU transducer.

The timing of our exposures was based on considerations pertinent to eventual use in a beating heart. Therefore, for in vivo studies, the exposure timing had to be synchronized with the cardiac cycle. The system software provided control over the duration and repetition rate used in exposure. It also incorporated electrocardiographic triggering system to synchronize exposures with the cardiac cycle (Figure 1, C). The HIFU beam was activated for 0.2 seconds starting at the R wave of the electrocardiographic signal at 2.0-second intervals. Ultrasound energy was delivered at an acoustic intensity of 23 kW/cm². Each dog received 6 sets of HIFU exposures of 20, 30, and 40 repetitions. In cardiac ablation applications, HIFU beam is easier to focus in LV wall during the end-systolic phase when heart motion is minimal and wall thickness is maximal. The duration of the end-systolic phase varies as a function of the heart rate. In our previous studies of this topic, the pulse duration was set to less than 0.3 seconds. We decided the repetition times as above because blood flow causes faster tissue cooling in vivo than occurred in our previous in vitro study.15

All studies were continuously recorded on super-VHS videotape, and video image clips of pre- and post-HIFU ablation were also stored digitally using video capture software (DVgate Plus, Version 1.0, Sony Corp, Tokyo, Japan) for subsequent offline analysis.

Within 1 hour after HIFU ablation the dogs were killed with an overdose of sodium pentobarbital (100 mg/kg intravenously). The hearts were removed and visible lesions were sectioned. Each lesion was fixed in 10% formalin and embedded in paraffin blocks. The tissue blocks containing the lesions were embedded in paraffin, and were sliced along the long axis of HIFU lesions, 5-µm thick. Myocardial sections were fixed onto glass slides and stained with standard hematoxylin-eosin and Masson trichrome.

The maximum lesion length, width, and depth from the endocardium of the septum to the lesion center were recorded using calipers (Figure 2) and the means and SD were computed. Lesion size measurements of the echocardiographic B-mode images were compared by two observers and compared with histopathologic lesions.

Statistical Analysis

Results are expressed as mean values ± 1SD. The lesion area size was calculated as 0.785 × length × width because the lesions resembled ellipsoid shape. Two-tailed paired Student t test was used for comparisons between the histologic and echocardiographic measurements. The intraclass correlation coefficient (ICC) was calculated as a measure of consistency between the histologic and echocardiographic measurements. Agreement among observers was also expressed according to the ICC. Statistical significance was defined as a P value of less than .05 for all analyses.

RESULTS

Evaluation of HIFU Lesion Variables

All lesions except one were clearly visible and well demarcated. One lesion was not detected because of equipment malfunction. The measured lesion lengths, widths, and areas by histologic and echocardiographic measuring method are summarized in Table.

During the HIFU exposures, premature ventricular contractions were sometimes observed but no fatal arrhythmias, such as ventricular tachycardia and ventricular fibrillation, occurred.

Histopathologic Evaluation of HIFU Lesions

Figure 2, A, shows a representative example of a myocardial section in the LV septum wall and Figure 2, B, shows an example of HIFU lesion stained with Masson trichrome and demonstrates the histopathologic features. The HIFU lesions appeared hyperesinophilic because of clumps of cytoplasmic materials. In the center of HIFU lesions, the myocardial cells were completely collapsed. However, outside the focal region, the myocardial tissue in the path of the HIFU beam was entirely intact.

Echocardiographic Evaluation of HIFU Lesions

All lesions except one were visible during HIFU exposures using echocardiography (Figure 3). HIFU lesions were visible as a high-echoic elliptical tissue and the lesion size increased gradually.

For graphic representations, the scatter plots of lesion area and depth are shown in Figures 4 and 5 together with the line of unity. The ICC was found to be 0.72, 0.63, 0.75, and 0.73 for lesion length, width, area, and depth, respectively. There was significant correlation between the actual histologic areas and those calculated from the echocardiographic images (ICC = 0.75). However, the echocardiographic lesion area and width were significantly bigger than the histologic ones (P < .005 and
There were no significant differences between the histologic and echocardiographic lesion lengths and depths ($P = .36$ and $P = .06$, respectively).

Analysis of calculated echocardiographic area between observers showed good agreement and ICC was found to be 0.88.

### Table  Number of HIFU exposure

<table>
<thead>
<tr>
<th></th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>Total</th>
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<tr>
<td><strong>Histological lesion size</strong></td>
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<tr>
<td>Length (mm)</td>
<td>5.8 ± 1.6</td>
<td>6.7 ± 1.0</td>
<td>7.4 ± 1.6</td>
<td>6.6 ± 1.4</td>
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<tr>
<td>Width (mm)</td>
<td>4.0 ± 1.1</td>
<td>5.0 ± 1.2</td>
<td>5.9 ± 1.7</td>
<td>4.9 ± 1.4</td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>18.7 ± 8.3</td>
<td>26.3 ± 8.7</td>
<td>35.5 ± 15.7</td>
<td>26.3 ± 12.1</td>
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<tr>
<td>Depth (mm)</td>
<td>10.9 ± 3.4</td>
<td>10.1 ± 2.5</td>
<td>10.7 ± 2.1</td>
<td>10.5 ± 2.5</td>
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|                   |     |     |     |       |
| **Echocardiographic lesion size** |     |     |     |       |
| Length (mm)       | 5.9 ± 1.6 | 7.7 ± 1.4 | 7.0 ± 1.7 | 6.8 ± 1.6 |
| Width (mm)        | 5.0 ± 0.9* | 5.9 ± 1.0 | 6.8 ± 1.2 | 5.9 ± 1.2* |
| Area (mm²)        | 23.8 ± 9.2* | 35.8 ± 11.1 | 38.2 ± 14.9 | 32.3 ± 12.4* |
| Depth (mm)        | 11.9 ± 2.9 | 11.5 ± 3.5 | 10.9 ± 3.4 | 11.5 ± 3.0 |

* $P < .05$ for the comparison of histologic lesion with echocardiographic lesion.

Our results demonstrate that HIFU allows extracardiac ablation of the LV septum in a beating canine heart. HIFU was able to produce well-demarcated thermal lesions and 2-dimensional diagnostic ultrasound image allowed precise ablation for in vivo...
Creation of thermal lesions was very consistent and reproducible. Feasibility of Echocardiographic Monitoring The HIFU lesion formation was visible using a diagnostic imaging transducer. The lesions were observed as highly echoic areas in the B-mode image. Just after the HIFU ablation, many microbubbles result from tissue boiling or cavitation\(^2\) and these microbubbles strongly reflect the ultrasonic beam. A gradual increase in lesion area was observed as the number of exposures increased. Echocardiographic monitoring during high-intensity focused ultrasound (HIFU) exposures. High echoic lesions were observed and lesion areas increased gradually.

Figure 3 Intraclass correlation coefficients (ICC) between echocardiographic and histologic lesion length (A) and width (B).
raphy will be useful for monitoring the HIFU exposure if these points are taken into consideration.

There was good correlation between the echocardiographic and histologic measurements. However, the measurements of lesion area from the echocardiography images were significantly larger than those measured from the histologic data because of significant differences in the apparent lesion width. The diffusion of the microbubbles by blood or intercellular fluid along the cardiac muscle layer might lead to this phenomenon.

**Clinical Implication of HIFU Ablation**

The results of this study demonstrate that targeted myocardial lesions may be created in vivo using HIFU without injuring endocardial or epicardial surfaces of the myocardium. The histopathology of the HIFU lesions confirmed the ability of HIFU to create definitive myocardial tissue destruction by thermal protein denaturation.

The extent of tissue injury and coagulative necrosis induced by HIFU varies linearly with exposure duration, exposure number, and acoustic intensity. In this study, we applied repeated 0.2-second exposures at multiple locations on each canine heart. If smaller or larger lesions are required, they may be produced by varying these exposure parameters. In addition, the HIFU ablation zone may be positioned by adjusting the location of the focal point with the assistance of a diagnostic transducer mounted in the HIFU transducer.

Because a HIFU system will not require a thoracotomy and is able to create the targeted lesions without injuring intervening tissues, HIFU may be less invasive than other techniques. HIFU systems may also allow us to reduce the required area for myocardial ablation. Our results indicate that HIFU may have a potential to reduce the LV wall thickness by myocardial ablation or to ablate arrhythmia foci and circuits. Ultrasound energy offers a potential alternative to current methods such as radiofrequency ablation to create therapeutic and focal myocardial lesions. This consideration is clinically relevant because structures that trigger arrhythmias can be located at any depth within the myocardium from the endocardial to the epicardial side. As described above, HIFU may have additional potential for LV septal ablation of hypertrophic obstructive cardiomyopathy.

**Limitations**

This technique has some limitations at this time. First, the focal depth of our transducer is 35 mm and then makes it difficult to create a lesion from outside the body. Second, because the diameter of our transducer is 33 mm, it is too large to aim the target...
tissue through the intercostal space and it is highly possible that the ribs reflect lateral HIFU beam and the ultrasound energy is attenuated. Moreover, the reflected HIFU beam would heat up and blow out the surface of HIFU transducer. However, when the transducer is developed, the focal depth becomes adjustable in the future; HIFU ablation without thoracotomy will be available and can be evaluated.

Conclusion

HIFU can be used to create targeted, well-demarcated thermal lesions in the LV septal myocardium during cardiac contraction and the HIFU lesion formation can be visible using a diagnostic imaging transducer. HIFU ablation may prove useful for noninvasive intramyocardial ablation.

REFERENCES