Feasibility of Targeting Atherosclerotic Plaques by High-Intensity–focused Ultrasound: An In Vivo Study

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ABSTRACT

Purpose: To investigate the feasibility and acute safety of targeting atherosclerotic plaques by high-intensity–focused ultrasound (US) in vivo through a noninvasive extracorporeal approach.

Materials And Methods: Four swine were included in this prospective study, three of which were familial hypercholesterolemic swine. The procedure was done under general anesthesia. After US identification of atherosclerotic plaques within the femoral arteries, plaques were targeted by high-intensity focused US with an integrated dual-mode US array system. Different ablation protocols were used to meet the study objectives, and animals were then euthanized at different time points. Targeted arterial segments were stained by hematoxylin and eosin for histopathologic examination. Numeric values are presented as means ± standard deviation.

Results: All swine tolerated the procedure well, with no arterial dissection, perforation, or rupture. Discrete lesions were detected in the first two swine, measuring 0.54 mm ± 0.10 and 0.25 mm ± 0.03 in cross-sectional dimensions in the first and 0.50 mm ± 0.12 and 0.24 mm ± 0.15 in the second. Confluent ablation zones were identified in the last two swine, measuring 6.92 mm and 0.93 mm in the third and 2.97 mm and 2.52 mm in the fourth. Lesions showed necrotic cores and peripheral reactive inflammatory infiltration. The endothelium overlying targeted arterial segments remained intact.

Conclusions: The results demonstrate the feasibility and acute safety of targeting atherosclerotic plaques by high-intensity–focused US in vivo. Further long-term studies are needed to assess how induction of these lesions can modify the progression of atherosclerotic plaques.

ABBREVIATION

H&E = hematoxylin and eosin

Atherosclerosis is a disease that affects large and medium-sized arteries (1). Available treatment options include conservative and interventional treatments depending on the degree of luminal stenosis and patient’s symptomatology (2). Interventional procedures like carotid endarterectomy and angioplasty still carry the risk of causing distal embolism, stroke, or even death during the procedure (3). On the contrary, the growth dynamics of atherosclerotic plaques that entail neovascularization and buildup of lipid-rich core (1) suggest that a plaque may respond to localized thermal therapy. A noninvasive form of thermal therapy capable of producing thermal necrosis within the plaque while preserving the overlying endothelium may introduce a new approach to interfere with the progressive growth of atherosclerotic plaques.

High-intensity–focused ultrasound (US) has been shown to produce localized thermal lesions and is currently used for clinical medical applications. The main mechanism of high-intensity focused US–induced tissue ablation is through the thermal effect, but other modes of high-intensity focused US tissue interactions are currently being investigated (4,5).
Dual-mode US array systems refer to advanced high-intensity focused US transducers that are capable of imaging and treating simultaneously by using the same elements. The same beam-forming is used to focus the dual-mode US array for imaging and therapy, which results in inherent registration between the imaging and therapy coordinate systems (6). This ensures accurate high-intensity focused US targeting and treatment monitoring. The use of dual-mode US arrays for this vascular application was based on successful targeting of blood vessels in previous experiments (7,8).

In the present study, we hypothesized that high-intensity focused US energy can be delivered with sufficient power by using a noninvasive extracorporeal approach to effectively induce thermal damage within arterial walls and atherosclerotic plaques in vivo. Four objectives were set for this study: (i) to test the feasibility and acute safety of inducing high-intensity focused US lesions within normal arterial walls, (ii) to validate the feasibility of inducing discrete high-intensity focused US lesions within atherosclerotic plaques by using various exposure parameters, (iii) to test the pattern of overlap of adjacent high-intensity focused US lesions within the plaques in an attempt to cover most of the volume of these plaques, and (iv) to report the resultant histopathologic changes within normal arterial walls and targeted plaques, paying special attention to the integrity of the overlying intimal endothelial lining as a safety determinant.

**MATERIALS AND METHODS**

**Study Design and Ablation Protocols**

The present study was approved by the institutional animal care and use committee. Four swine, three of which had familial hypercholesterolemia, were studied in this sponsored prospective feasibility study. Ablation protocols were set to meet the study objectives. For the first swine, three planes (7 mm apart) were targeted across the longitudinal axis of the healthy left femoral artery. Three discrete high-intensity focused US shots were placed on the anterior wall of the artery in each plane. The estimated focal intensity was approximately 4,800 W/cm², and exposure time ranged from 250 to 750 ms. For the second swine, five discrete high-intensity–focused US shots were placed at different locations along the atherosclerotic plaque in the left femoral artery, as identified by US scanning. Exposure time ranged from 500 to 2,000 ms, and the estimated focal intensity ranged from 4,700 to 5,600 W/cm². For the third animal, three high-intensity focused US shots (2,000 ms; 4,100-4,400 W/cm²) were placed adjacent to each other in the same plane across the atheromatous plaque in the right femoral artery. For the fourth animal, three planes (7 mm apart) were treated across the longitudinal axis of the left femoral artery. Seven adjacent high-intensity–focused US shots (2,000 ms; 4,100-4,400 W/cm²) were placed in each plane. Animals were euthanized at different time points after the procedure to explore the histologic changes developing over time. The first animal was euthanized shortly after the procedure. The second and third swine were killed on day 4. The fourth animal was euthanized on day 7 after the procedure. Numeric values are presented as means ± SD.

**High-intensity–focused US Transducer**

The dual-mode US array (Imasonic, Voray sur l’Ognon, France) used in the present study was an integrated 3.5-MHz, 64-element phased-array transducer with a focal length of 40 mm. The transducer has a central fenestration through which a linear 10-MHz diagnostic transducer (HST 15-8/20; Ultrasonix, Richmond, British Columbia, Canada) was applied (Fig E1, available online at www.jvir.org). This design provides complementary diagnostic imaging in addition to the inherent imaging capabilities of the dual-mode US array.

**Animal Model**

Familial hypercholesterolemic swine were used in this study as a result of the close similarity of disease pathogenesis and pattern of plaque progression as that in humans (9–13).

**Experimental Setup**

The procedures were performed under general anesthesia. Animals were fasted for at least 12 hours before the procedure. Induction of anesthesia was done with Telazol (xylazine; Pfizer, Olot, Spain)/Xylazine (Llyod Laboratories, Shenandoah, Iowa) (2–8 mg/kg). Maintenance anesthesia was achieved by using IsoFlurane (Piramal Healthcare Limited, Andhra Pradesh, India; 100% O₂, 0%–5% inhalation) inhalation through endotracheal tube. All vital signs were monitored and recorded throughout the procedure.

Swine were placed in supine position. Preliminary diagnostic US scanning (Sonix RP; Ultrasonix, Richmond, British Columbia, Canada) was performed by using a 7.2-MHz transducer (L14-5/38; Ultrasonix) to evaluate the atherosclerotic burden in both hindlimb arteries. Nonulcerated atheromatous plaques were detected along the left femoral artery of the second and fourth swine, as well as the right femoral artery of the third swine, whereas no atherosclerotic plaques were seen in the first swine (healthy swine). Detected plaques caused no significant hemodynamic compromise (Fig E2, available online at www.jvir.org).

When plaques had been detected and characterized, skin over the corresponding groin was shaved and cleaned thoroughly to remove any dirt. Moreover, gentle traction of the skin was applied by using metallic clips attached to the skin to overcome any skin wrinkles that may entrap gas microtubules. Such microtubules may interfere with the passage of the high-intensity focused US therapeutic beam.
A detailed diagnostic US scan was then performed in two perpendicular planes to determine the point for best visualization of the atherosclerotic plaque. Two orthogonal lines intersecting at this point were marked on the skin for initial alignment of the dual-mode US array. This point marked the ideal entry position for targeting the plaque by using the dual-mode US array (Fig 1a).

A water bolus was used to couple the transducer assembly to the target tissue, taking care to avoid the formation of gas bubbles in the path of the high-intensity-focused US beam. To ensure good coupling, a thin membrane attached to circular metallic frame was sealed to the skin surface by using veterinary glue. When the glue had cured, degassed water was added and contained within the membrane. Finally, the dual-mode US array was centered over the aforementioned entry point (Fig 1b).

By using the integrated dual-mode US array system, three imaging modes were used to guide and monitor the procedure. Conventional diagnostic imaging (conveyed through the diagnostic transducer within the fenestration) was used to visualize the artery and the plaque and to collect data regarding the depth of the artery, arterial diameter, and thickness of the subcutaneous fat layer. Two-dimensional M-mode imaging could detect the location of the arterial pulsations along the axial and lateral directions and was used to monitor changes at the focal point during ablation. Two dual-mode US array imaging modes (7, 8) were used in these experiments: (i) synthetic aperture imaging was used to visualize the artery by using the same elements that generate the therapeutic high-intensity focused US beam and (ii) single-transmit focus was used to monitor the tissue response before, during, and after the high-intensity-focused US shot at frame rates greater than 100 frames per second. The inherent registration between the imaging and therapy coordinate systems is an important advantage of imaging with dual-mode US arrays (Fig 2).

After each shot, the therapeutic process and the condition of the artery were evaluated by using the three mentioned imaging modes. For all animals, additional high-intensity focused US shots were delivered in the muscles in vicinity of the targeted arterial segments to mark the treatment planes. These “soft-tissue markers” facilitated identification of the targeted segments during tissue trimming and histologic evaluation (Fig E3, available online at www.jvir.org).

On finishing the procedure, animals were transferred to a recovery room and monitored closely during the recovery period. Animals were then euthanized at the predetermined times.

**Histologic Processing and Evaluation**

All swine underwent necropsy by trained staff at the test facility. The proximal end of the targeted arteries were exposed and perfused with lactated Ringer solution followed by 10% neutral buffered formalin for at least 10 minutes. Then, the entire en-bloc section of the artery and the surrounding muscles was harvested, including 1 cm of the untreated proximal vessel and 1 cm of untreated distal vessel. A label was sutured to the proximal end of each vessel and placed in 10% neutral buffered formalin.

After fixation, treated vessels were sectioned through the entire vessel length. Sections believed to contain targeted plaques were determined by the study pathologist, placed in cassettes, and left in a tissue processor.
for paraffin infiltration. Samples were then serial sectioned through the entire block at 200-μm intervals. Slides were then further cut at 4 μm and stained with hematoxylin and eosin (H&E) stain. All tissue slides were evaluated by the study pathologist. Photographs were taken for every step of the procedure.

RESULTS

All swine tolerated the procedure well, with no hemodynamic instability or significant changes in vital signs. No arterial dissection, perforation, or rupture occurred during or after high-intensity focused US shooting. The radiation force of the high-intensity focused US beam caused the arteries to minimally displace dorsally during high-intensity focused US shooting. However, upon stoppage of high-intensity focused US shooting, arteries returned back to their original position, retaining normal pulsatility and blood flow. No signs of disability were reported during the recovery period and animals showed perfect recovery until the day of euthanasia.

Histologic examination of the targeted arteries showed the following findings. For the first swine, four discrete high-intensity–focused US lesions were identified (Fig 3). Lesions consisted of small shrunken and hypereosinophilic smooth muscle cells with small dark nuclei...
and increased amounts of myxomatous matrix within tunica media. The mean length and width of the lesions were 0.54 mm ± 0.10 and 0.25 mm ± 0.03, respectively, and the mean area was 0.109 mm² ± 0.026. For the second swine, five discrete lesions were identified within the substance of the atherosclerotic plaque, consistent with the five high-intensity focused US shots (Fig 4). The mean length and width of the lesions were 0.50 mm ± 0.12 and 0.24 mm ± 0.15, respectively. The mean lesion area was 0.095 mm² ± 0.052.

For the third swine, a focal area of mural hemorrhage was identified on gross pathologic examination of the treated artery, corresponding to the presumed area of high-intensity focused US ablation (Fig E4, available online at www.jvir.org). On histologic evaluation, three lesions consistent with acute thermal injury were identified. However, because the three lesions were placed adjacent to each other, and as a result of the heat conduction within the substance of the plaque, lesions partly overlapped, forming a confluent ablated zone measuring approximately 6.92 mm, 0.93 mm, and 4.93 mm² in length, width, and area, respectively (Fig 5).

For the fourth swine, confluent high-intensity focused US thermal lesions were identified in the posterior wall of the femoral artery in treatment planes I and III (Fig E5, available online at www.jvir.org). The confluent ablation zone in plane III measured approximately 2.97 mm, 2.52 mm, and 7.62 mm² in length, width, and area, respectively. There was unequivocal arterial mural injury within the posterior wall in treatment plane II, but it was not determined whether this injury was a consequence of the natural degeneration within the atherosclerotic plaque or a result of high-intensity–focused US thermal injury. There were two additional findings in this animal. First, there was an instance of minimal mural thrombus formation in the posterior wall of the targeted left femoral artery in plane III. This minute focus of thrombosis was incorporated within
the vascular wall (ie, normal healing process) and completely recovered by complete endothelialization (Fig 6a, 6b). Second, there was evidence of thrombus formation within the neovessels/vasa vasorum of the treated arterial segment in plane II (Fig 6c). However, whether thrombus formation was a consequence of high-intensity focused US treatment was undetermined.

High-intensity–focused US-induced thermal injury within atherosclerotic plaques showed distinct histologic features (Fig 4b and Figs E6, E7, available online at www.jvir.org). Targeted plaques showed necrotic cores containing cellular debris. The periphery of the lesions was infiltrated by neutrophils and other inflammatory cells indicating reactive inflammatory process. Some of the treated plaques showed foci of hemorrhage within. There was no evidence of microscopic injury of the endothelium lining the targeted healthy arterial wall (first swine) or the atherosclerotic plaques (swine 2-4). For the single instance in which a minute mural thrombus was noted (swine 4, plane III), the thrombus was completely covered by intact smooth endothelium.

DISCUSSION

Thermal therapy is in use or under investigation for treatment of occlusive vascular diseases. For example, it was shown that cryoplasty improves results of percutaneous transluminal angioplasty by limiting the incidence of dissection, restenosis, and need for stent placement (14). On the contrary, research studies showed that hyperthermia reduced inflammatory infiltration of arterial walls and suppressed neointimal hyperplasia (15). These changes may have a beneficial role against progression of atherosclerosis. For vascular applications in general, and treatment of atherosclerotic plaques specifically, safety considerations take priority. Special attention was paid to the safety of this potential new application. In the present study, a total of 38 high-intensity focused US shots were performed at different locations along the femoral arteries by using different exposure levels. No arterial rupture, dissection, or perforation was observed within the range of the exposure levels used.

The safety determinants were extended beyond gross anatomic evaluation to include histologic analysis. One of the primary considerations in designing the exposure levels was to minimize direct injury or sloughing of the intimal endothelial lining covering the targeted plaques. Intimal endothelial injury, if it had happened, may have been considered a serious limitation for this potential application, as the exposed subendothelial collagen may initiate platelet plug formation and thrombotic cascade that may end in thrombus formation. Such local thrombosis may aggravate the existing luminal narrowing and may carry the risk of distal embolism. Histologic evaluation showed that the endothelium covering the treated plaques remained intact and smooth for all the treated plaques. Even for the single case in which a subendothelial minute thrombus was detected, it showed complete endothelialization with no evidence of intraluminal thrombus extension. In addition, histologic evaluation showed that high-intensity focused US lesions occurred in the core of the plaque whereas the cap of the plaque was left intact. This could be significant from a safety standpoint. The fact that the cap was left intact whereas the nearby plaque tissue suffered thermal damage portends well for adequate treatment with low risk of distal embolism. The fact that the endothelium remained intact despite the damage within the plaque can be explained by the heat-sink effect of the flowing blood. This is in contrast to the core of the plaque, with its high lipid content, that tends to trap and accumulate more thermal energy sufficient to induce thermal damage within plaque substance.

It is noteworthy that, for the first swine (ie, the healthy one), only four lesions were detected among nine shots.
This observation has several possible explanations. First, the high-intensity focused US transducer had to be moved from one plane to another to perform the high-intensity focused US shots. Even though the movement was trivial, minimal changes in subcutaneous fat thickness and degree of transducer angulation relative to the artery may have contributed to increased prefocal attenuation of the high-intensity focused US beam. This may have decreased the delivered therapeutic dose below the threshold for thermal damage. Although the protocol used in swine 2 (with familial hypercholesterolemia) included similar movement, the target plaque tissue may have reacted differently to the same level of exposure because the fat content of the plaques can retain more high-intensity focused US energy, making plaques more susceptible to high-intensity focused US damage than the relatively resistant normal arterial walls. Second, as the first animal was euthanized shortly after the procedure, the reactive inflammatory changes at the targeted segments may have not had enough time to develop and register on histologic examination by the time of euthanasia. Third, the possibility of having missed some of the lesions on histologic analysis (because of their minute sizes) could not be ruled out.

It was noticed that the shape and size of high-intensity–focused US lesions is not the same, even after the same exposure levels. This raises the possibility that the plaque substance itself may be an important determinant of the configuration of the induced high-intensity focused US lesion. This also calls for improved feedback control of lesion formation and/or treatment planning to minimize this variation.

The present study is not without limitations. Although dual-mode US array imaging conveyed essential information for guidance and monitoring of the process, there were instances when the dual-mode US array imaging quality was inadequate. This was primarily because of US reverberation within the water bolus between the concave array and the skin. This reduced the contrast between the target vessel wall and its lumen on dual-mode US array imaging, which made targeting difficult at times. However, the effects of US reverberations can be mitigated by the use of coded excitation (16). Another limitation was the difficulty in identifying high-intensity–focused US lesions on histologic examination because of the minute size of these lesions and paucity of published data regarding histologic characterization of high-intensity focused US damage in arterial walls or atherosclerotic plaques. It was somewhat challenging to locate the induced high-intensity focused US damage at first. However, with time, the study pathologist became familiar with the appearance of such lesions and was able to identify the damage with confidence. All the histology sections were evaluated by the same pathologist under the same standards and in view of the accumulated experience in identifying the high-intensity–focused US-induced damage.

Results showed the feasibility of using high-intensity–focused US, through a completely noninvasive extracorporeal approach, to induce thermal lesions within atherosclerotic plaques. However, the long-term goal is to explore if such lesions can interfere with the natural progression of atherosclerotic plaques. As plaque growth entails a cascade of recruitment of inflammatory cells, smooth muscle hyperplasia, and neovascularization (1), it will be important to see how the induced damage can interfere with this process in a way that may slow down or even interrupt the course of the disease. Further long-term studies will be needed to investigate such a hypothesis.

In conclusion, the present study demonstrates the feasibility of using image-guided high-intensity focused US in forming localized lesions within plaque tissues in peripheral vessels without damage to the endothelium. If this approach were proven to reverse the progression of plaques in humans, it may provide lower-risk options for specific patient populations. For example, asymptomatic patients who have localized plaques discovered incidentally on duplex scanning may benefit from this application. These patients may receive high-intensity–focused US for their localized plaques in addition to lipid-lowering drugs. The results are also relevant to other applications in which targeting the tissue surrounding arterial walls for ablation has medical significance. For example, renal denervation is a promising application for thermal therapy that could be safely administered by using dual-mode US array–guided high-intensity focused US described in the present study.

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REFERENCES


Figure E1. Integrated dual-mode US array system: 3.5-MHz dual-mode US array transducer with a central fenestration through which a linear diagnostic transducer is applied.

Figure E2. Longitudinal US scan of the left femoral artery of swine 4 with a 7.5-MHz transducer shows a non-hemodynamically significant atherosclerotic plaque along the posterior wall of the artery (arrows).

Figure E3. Muscle marker. H&E staining of a skeletal muscle shows evidence of muscle injury in the form of moderate variation in skeletal cells diameter, a thickened or fibrotic perimysium, vacuolation of muscle fibers, tinctorial changes in eosinophilic staining, separation of muscle cells from surrounding endomysium, and multiple nuclei not located at the cell periphery.

Figure E4. Focal hemorrhage within targeted atherosclerotic plaque (from swine 3). Gross pathologic evaluation of the targeted arterial wall shows focal mural hemorrhage (arrow). No luminal thrombosis identified.
Figure E5. High-intensity–focused US-induced thermal injury within atherosclerotic plaque (from swine 4). H&E staining (objective magnification, × 2) of the targeted plaque shows inflamed atherosclerotic plaque secondary to thermal injury, with inflammation extension into the subjacent tunica media.

Figure E6. Histologic examination of high-intensity–focused US-induced thermal damage within atherosclerotic plaque (from swine 4). H&E staining of the targeted plaque shows hemorrhage within the targeted plaque and presumptive central area of thermal necrosis.

Figure E7. Histology of high-intensity–focused US-induced thermal damage within atherosclerotic plaque. H&E staining of the targeted plaque shows an area of thermally altered extracellular lipid or thermal necrosis of lipid-laden macrophages.