Published in final edited form as:

J Vasc Interv Radiol. 2012 July ; 23(7): 953–961.e2. doi:10.1016/j.jvir.2012.04.001.

Pulsed High–Intensity-focused US and Tissue Plasminogen Activator (TPA) Versus TPA Alone for Thrombolysis of Occluded Bypass Graft in Swine

Nadine Abi-Jaoudeh, MD,

Department of Radiology and Imaging Sciences, National Institutes of Health, Bethesda

William F. Pritchard, MD, PhD,

Laboratory of Cardiovascular and Interventional Therapeutics, Center for Devices and Radiological Health, United States Food and Drug Administration, Laurel

Hayet Amalou, MD,

Department of Radiology and Imaging Sciences, National Institutes of Health, Bethesda

Marius Linguraru, DPhil,

Sheikh Zayed Institute for Pediatric Surgery Innovation, Children's National Medical Center, Washington, DC

Oscar A. Chiesa, DVM, PhD,

Laboratory of Cardiovascular and Interventional Therapeutics, Center for Devices and Radiological Health, United States Food and Drug Administration, Laurel

Joshua D. Adams, MD,

Departments of Vascular Surgery and Radiology, University of Virginia, Charlottesville, Virginia

Carmen Gacchina, PhD,

Department of Radiology and Imaging Sciences, National Institutes of Health, Bethesda

Robert Wesley, PhD,

Office of the Director, Clinical Center, National Institutes of Health, Bethesda

Subha Maruvada, PhD,

Offices of Science and Engineering Laboratories, Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, Maryland

Briana McDowell, BA,

Laboratory of Cardiovascular and Interventional Therapeutics, Center for Devices and Radiological Health, United States Food and Drug Administration, Laurel

Victor Frenkel, PhD,

Department of Biomedical Engineering, Catholic University of America

John W. Karanian, PhD, and

© SIR, 2012

Address correspondence to N.A.-J., Department of Radiology and Imaging Sciences, National Institutes of Health, Room 1C365, Building 10, MSC 1182, 9000 Rockville Pike, Bethesda, MD 20892; naj@mail.nih.gov.

The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as an actual or implied endorsement of such products by the US Food and Drug Administration, the National Institutes of Health, the Department of Health and Human Services, or the Public Health Service.

None of the authors have identified a conflict of interest.

This article includes an Appendix that is available online at www.jvir.org.

Laboratory of Cardiovascular and Interventional Therapeutics, Center for Devices and Radiological Health, United States Food and Drug Administration, Laurel

Bradford J. Wood, MD

Department of Radiology and Imaging Sciences, National Institutes of Health, Bethesda

Abstract

Purpose—Prosthetic arteriovenous or arterial-arterial bypass grafts can thrombose and be resistant to revascularization. A thrombosed bypass graft model was created to evaluate the potential therapeutic enhancement and safety profile of pulsed high-intensity-focused ultrasound (pHIFU) on pharmaceutical thrombolysis.

Materials and Methods—In swine, a right carotid-carotid expanded polytetrafluoroethylene bypass graft was surgically constructed, containing a 40% stenosis at its distal end to induce graft thrombosis. The revascularization procedure was performed 7 days after surgery. After model development and dose response experiments ($n = 11$), two cohorts were studied: pHIFU with tissue plasminogen activator (TPA; $n = 4$) and sham pHIFU with TPA ($n = 3$). The experiments were identical in both groups except no energy was delivered in the sham pHIFU group. Serial angiograms were obtained in all cases. The area of graft opacified by contrast medium on angiograms was quantified with digital image processing software. A blinded reviewer calculated the change in the graft area opacified by contrast medium and expressed it as a percentage, representing percentage of thrombolysis.

Results—Combining pHIFU with 0.5 mg of TPA resulted in a $52\% \pm 4\%$ increase in thrombolysis on angiograms obtained at 30 minutes, compared with a $9\% \pm 14\%$ increase with sham pHIFU and 0.5 mg TPA ($P = .003$). Histopathologic examination demonstrated no differences between the groups.

Conclusions—Thrombolysis of occluded bypass grafts was significantly increased when combining pHIFU and TPA versus sham pHIFU and TPA. These results suggest that application of pHIFU may augment thrombolysis with a reduced time and dose.

Prosthetic grafts have several clinical applications but are used mainly as arteriovenous shunts for hemodialysis and as bypass grafts to treat peripheral vascular disease. Graft occlusion can occur in as many as 50% of lower-extremity bypass grafts (1). Several factors contribute to bypass graft failure: the inherent thrombogenicity of the graft caused by the lack of endothelial cell lining, mismatch between graft size and native vessel, and low flow associated with smaller grafts (ie, diameter < 6 mm) (2). Catheter-directed thrombolysis is the recommended initial treatment according to the Trans-Atlantic Inter-Society Consensus (3) and is generally well accepted. However, the potential to improve lytic efficacy and reduce time and dose associated with pharmacologic thrombolysis have fueled interest in adjunctive therapies (3,4). Ultrasound (US)-enhanced thrombolysis is one example; both catheter-delivered (ie, endovascular) and externally applied (ie, transcutaneous) US systems exist (5,6).

US is defined in part by its frequency and acoustic intensity parameters. Low frequency ranges are assigned between 20 and 400 kHz and high frequency ranges between 0.5 and 3 MHz. US intensity is defined as low when tissue exposure is less than 2 W/cm^2 and high if greater than 2 W/cm^2 (7). US parameters are mutually linked (7). Low-frequency US waves penetrate deeper in tissue without loss of energy whereas high-frequency US waves require higher intensities to achieve a similar effect at a given depth. These higher intensities delivered in continuous manner result in tissue heating (7,8); however, intermittent or pulsed high-intensity-focused US (HIFU; pHIFU) sonication lowers the rate of energy deposition and diminishes tissue heating (7,9). Commercially available tools currently consist of

catheter-delivered intravascular low-intensity US (10,11); therefore, the beam is not focused and tissue exposure is low. This tool has shown some clinical benefit in the setting of peripheral arterial and venous thrombosis (5,8,12,13), and randomized studies are currently under way (13). Low-intensity focused US in conjunction with microbubbles has been shown to rapidly dissolve thrombi without lytic agents in swine and dog models (14–16). The theoretical mechanism of action is cavitation (17). However, the use of microbubbles may have regulatory implications impeding translation to clinic and can have undesirable side effects, mainly soft-tissue injury (17). One advantage of HIFU is the ability to generate a small focus area (6). Preliminary data with the use of pHIFU to enhance pharmacologic thrombolysis in vitro and in small animal models are encouraging (18,19). pHIFU is a noninvasive external focused high-intensity beam, which is applied to the treatment area in a defined time frame (1 minute per sonication point), unlike current commercial technologies that require catheterization of the vessel and continuous delivery of a low-intensity beam over a period of several hours (10,11).

The goals of the present study were to develop a large animal model of bypass graft thrombosis, determine the dose response of tissue plasminogen activator (TPA) in 7-day-old thrombus, and evaluate the safety and effectiveness of thrombolysis in conjunction with pHIFU. This study examined the hypothesis that pretreating a thrombosed arterial bypass graft with pHIFU, followed by the administration of a commonly used thrombolytic agent such as TPA, would enhance thrombolysis compared with the thrombolytic agent alone, thereby reducing the thrombolytic agent dose needed.

Materials and Methods

Study Endpoints and Design

The primary endpoint was the degree of thrombolysis assessed by angiography in 7-day-old clot following pHIFU or sham pHIFU and TPA. The secondary endpoint was safety of the therapy, specifically damage to the graft or surrounding tissue. The study was performed under an animal use protocol approved by the appropriate institutional animal care and use committee. Detailed description of methodology, in particular the HIFU device and exposure, pathologic analysis, and image analysis, is provided in the Appendix (available online at www.jvir.org).

HIFU Device and Exposures

The therapeutic transducer (Focus Surgery, Indianapolis, Indiana) was a 1–3 piezocomposite material designed as a concave bowl (90-mm diameter) with a 37-mm coaxial opening and rubber bushing for the US imaging transducer (C8-5 8- to 5-MHz broadband curved array; Philips, Bothell, Washington) that provides real-time guidance. The spherically focused transducer had a focal length (ie, radius of curvature) of 10 cm. The US frequency was 1 MHz.

The acoustic output of the therapeutic transducer was measured. With the indicated system power set at 90 W, the measured acoustic power during the pulse is 71.3 W. The focal field of the therapeutic transducer was characterized with a calibrated hydrophone (Onda, Sunnyvale, California). To obtain a more accurate assessment of the actual in situ acoustic pressure at the focus within the tissue, simulations were performed. The values of the peak positive and peak negative pressures at the focus were 7.4 MPa and 4.2 MPa, respectively. The spatial-peak intensity during the pulse at the focus was 857 W/cm².

Bypass Graft Model Development

The study used 18 castrated male domestic swine (Archer Farms, Darlington, Maryland) with a mean weight of 188 lbs (range, 110–250 lbs). Initial sedation was achieved with a combined intramuscular injection of xylazine (Rompun; Phoenix Pharmaceutical, St. Joseph, Missouri) and zolazepam HCL (Telazol; Fort Dodge Animal Health, Overland Park, Kansas) at a dose of 1–3 mg/kg each in a solution containing 100 mg/mL of each, followed by intravenous administration of 0.020–0.024 mL/kg of a solution of xy-lazine (29 mg/mL), zolazepam HCL (15 mg/mL), ketamine HCL (59 mg/mL; Lloyd Labs, Shenandoah, Iowa), atropine sulfate (0.88 mg/mL; Neogen, Lexington, Kentucky), and butorphanol tartrate (0.59 mg/mL; Torbugesic; Wyeth, Madison, New Jersey). Following endotracheal intubation, general anesthesia was maintained with isoflurane 2%–3% (Allianz Surgical, Coconut Creek, Florida). No paralytic agents were used.

An ipsilateral right carotid-carotid bypass was performed by using the expanded polytetrafluoroethylene prosthesis (Vascutek; Terumo, Ann Arbor, Michigan) with a proximal end-to-end anastomosis, a distal end-to-side anastomosis, and ligation of the intervening native carotid artery. To induce thrombosis, a stenosis with approximately 40% diameter reduction was constructed at the distal end of the graft with a suture (Fig 1). Occlusion was confirmed by palpation or US. After the surgical procedure, the animals were allowed to recover from anesthesia.

The thrombolysis studies were performed after the thrombus had matured for 7–8 days, and were conducted under general endotracheal anesthesia. A 5-F sheath (Avanti; Cordis/Johnson and Johnson, Piscataway, New Jersey) was placed in the left jugular vein and a computed tomographic (CT) angiogram of the neck was obtained in the arterial phase during intravenous injection of iodinated contrast medium (Isovue 300; Bracco, Princeton, New Jersey) at a rate of 4–5 mL/s on a 16–detector row CT unit (MX8000/IDT; Philips, Cleveland, Ohio) with a field of view of 250 mm, slice thickness of 1 mm, and increment of 0.5 mm. US examination of the bypass graft was also performed. A 5-F sheath (Avanti; Cordis/Johnson and Johnson) was placed in the left internal carotid artery, through which a guide catheter (Cordis, Miami Lakes, Florida) was advanced to the origin of the brachiocephalic artery. A 4-F, 5-cm (infusion length) thrombolysis catheter (AngioDynamics, Queensbury, New York) was advanced into the right carotid-carotid bypass graft over a guide wire. Animals were anticoagulated with heparin.

The thrombolytic agent TPA (Activase; Alteplase; Genentech, South San Francisco, California) was used. Alteplase was supplied as a 50-mg vial of lyophilized powder that was reconstituted in 50 mL sterile water.

Dose Response Experiments

Determining angiographic thrombolysis of various doses of TPA alone was key to clarifying the contribution of pHIFU. Dose response experiments were performed in three animals by administering three doses of TPA: 0.25 mg, 0.5 mg, and 2 mg. For each dose, a prelytic angiogram was obtained (BV Pulsera; Philips). The TPA, followed by a 2-mL saline solution flush, was administered by pulse-spray technique over a period of 5 minutes. Serial angiograms at 10, 15, 30, and 60 minutes were obtained after TPA administration. There was a 90-minute interval between each dose (ie, > 10 times the half-life of TPA).

Therapeutic Interventions

Based on results of the dose response experiments, two cohorts were studied: animals that received pHIFU and 0.5 mg TPA (n = 4) and those that received sham pHIFU and 0.5 mg TPA (n = 3). When vascular access had been obtained and preliminary imaging had been

completed, the pulse-spray catheter was introduced into the bypass graft as described earlier, and a baseline angiogram was acquired. The HIFU transducer was positioned by using real-time US imaging provided by the diagnostic probe so that the graft was within the focal zone. Sixty-second exposures with a 10% duty cycle were performed at 5-mm intervals over a 5-cm section of the graft, for a total of 10 treatment points. The indicated power was set at 0 W for sham pHIFU and at 90 W for pHIFU. Otherwise, the two groups were treated identically.

When pHIFU or sham pHIFU application had been completed, an angiogram was obtained. Then, 0.5 mg TPA followed by a 2-mL saline solution flush was administered through the lysis catheter in a pulse-spray fashion over a period of 5 minutes. Follow-up angiograms were obtained at 10, 15, and 30 minutes after the initiation of TPA administration. All angiograms were obtained with the same imaging parameters (ie, frames per second and magnification) as the baseline angiogram.

After completion of the final angiography study, the animal was euthanized, and dissection en block of the right neck area was performed. Harvested tissues were stored in 10% formaldehyde solution. The specimens included the skin, subcutaneous tissue, muscles overlying the treatment area, as well as native right carotid artery and bypass graft. Pathologic analysis of the specimens was performed by a board-certified pathologist at an outside independent institution, who was blinded to treatment group.

Image Analysis

A blinded reviewer performed the analysis of the angiographic results by using digital image processing software (Medical Image Processing, Analysis, and Visualization; National Institutes of Health, Bethesda, Maryland).

For the sham pHIFU animals, the graft dimensions (width and length) were normalized for magnification and used to calculate the graft area in the image space. An interactive segmentation algorithm that uses edge detection (Livewire [20]) was used to trace the patent graft area represented by the area of graft opacified by contrast medium on the angiograms at each time point. The automated edge detector ensured a higher degree of reproducibility of the results compared with manual segmentation. The difference of patent graft area at a time point compared with the pretreatment angiogram (ie, Δ) represented the percentage of thrombolysis. The average intensity of pixels within the contrast area was also provided by the image analysis software. This was a negative image, meaning that higher contrast had lower pixel values.

Statistical Analysis

To test the hypothesis of the superiority of pHIFU, the sample size initially was calculated to require six animals per group by using a standard two-sample *t* test, with a two-sided level of significance (ie, α value) of 0.05 and power of 80% to detect an expected difference of 40% with a common SD of 20% (based on preliminary work in two animals). Because of the uncertainty in this estimate, we designed the study to include an interim analysis to determine whether a moderate increase in the sample size was required, according to the approach of Shun et al (21). This interim analysis does not inflate the α value; however, it does not allow the study to be ended early with a significant difference declared. However, at the interim analysis, a dramatic and unexpected difference was observed, and the study was prematurely terminated. As this deviated from the original design, our reported results have been adjusted by doubling the *P* value according to Bonferroni inequality, which reflects a penalty for early stoppage. Means $\pm$ SD are reported throughout, and all *P* values are two-sided.

Results

The total number of swine in the study was 18: six were needed for model development, three for dose response experiments, and nine for the main study; however, two animals were excluded.

Model Development

US examination of the native carotid artery before surgery confirmed vessel patency and size (6–7 mm). Surgery was technically successful in placing the bypass graft in all cases except for one animal that died of malignant hyperthermia.

Three animals had incomplete thrombus or patent grafts, which led to adjustment of the distal stenosis to ensure thrombosis. A few animals were noted to have hematomas, and one developed an abscess and recanalization of the graft. We subsequently modified the surgical technique with two small incisions to reduce infections and bleeding. An inability to catheterize the bypass graft necessitated the proximal anastomosis to be changed from end-to-side to end-to-end.

The 12 remaining animals were used for the dose response study ($n = 3$) and the main study ($n = 7$), and two animals intended for the sham HIFU group were excluded because of leaks from one of the anastomoses.

Two swine developed small hematomas around the graft diagnosed on CT angiography and US without clinical signs; these animals were not excluded from analysis because their bypass grafts remained thrombosed.

Dose Response Experiments

Analysis of the percentage of thrombolysis was calculated for each dose during dose response experiments. For the 0.25-mg dose of TPA, the mean thrombolysis percentages detected were each $0\% \pm 10\%$ at the 10-, 15-, and 30-minute angiograms ($n = 3$). The 0.5-mg dose of TPA ($n = 3$) yielded mean thrombolysis percentages of $6\% \pm 13\%$ at the 10-minute angiogram, $7\% \pm 10\%$ at 15 minutes, and $13\% \pm 9\%$ at 30 minutes. Finally, 2 mg of TPA ($n = 3$) produced mean thrombolysis percentages of $17\% \pm 15\%$, $21\% \pm 9\%$, and $27\% \pm 17\%$ on 10-, 15-, and 30-minute angiograms, respectively. The dose selected for the therapeutic study was 0.5 mg because it was just greater than the threshold for angiographically detectable thrombolysis in which the potential effects of pHIFU could be best detected.

Study Endpoints

US and CT angiography performed the day of the revascularization procedure confirmed complete thrombosis of the graft except in four cases as described earlier. There was no difference in stenosis between the pHIFU and sham pHIFU groups, with the area reductions measuring $60\% \pm 0.5\%$ and $59\% \pm 0.4\%$ for pHIFU and sham pHIFU, respectively ($P = .7$).

The percentages of patent graft area on pretreatment angiograms averaged $20.7\% \pm 19.5\%$ for the pHIFU/TPA group and $12.8\% \pm 15\%$ for the sham pHIFU/TPA group ($P = .58$). The percentages of patent graft area on angiograms obtained 30 minutes after lysis were $72.5\% \pm 17.6\%$ for the pHIFU/TPA group and $21.8\% \pm 10.2\%$ for the sham pHIFU/TPA group ($P = .007$; Fig 2). More importantly, the 30-minute percentage of thrombolysis (ie, Δ) was $51.8\% \pm 3.5\%$ for the pHIFU/TPA group, compared with $9.1\% \pm 13.7\%$ for the sham pHIFU/TPA group ($P = .003$, Bonferroni adjusted; Fig 3). The Table details the percentage of thrombolysis based on angiographic results per group at each time point. The average pixel

intensity within the contrast areas at 30 minutes was similar between the two groups ($P = .86$, Bonferroni adjusted).

Pathologic analysis revealed no significant differences between the two groups in terms of skin or graft damage, inflammation, and infection (Fig 4). Normal postoperative changes were noted in all submitted samples ($n = 7$) as demonstrated by reactive fibrovascular tissue with some evidence of hemorrhage and/or proteinaceous fluid accumulation. A collar of proliferating fibrovascular tissue surrounding the graft was graded as moderate (grade 3; Appendix, available online at www.jvir.org) in all specimens. Accumulation of fluid and fibrin surrounding the graft was qualitatively graded as marked (grade 4) in two pHIFU and one sham pHIFU case, and as moderate in two pHIFU and two sham pHIFU cases. Dermatitis graded mild to moderate (grades 2/3) was observed in three animals in the pHIFU group and two animals in sham pHIFU group. There was no evidence of necrosis or burns to the skin or subcutaneous or perigraft tissues. Erythrocytes and proteinaceous fluid were noted to have accumulated within the fibers of grafts similarly (grade 2/3) in the sham pHIFU and pHIFU groups (Fig 5).

Discussion

Catheter-directed thrombolysis is generally well accepted; however, success rates for thrombotic and embolic occlusions in the lower extremities average 81% and 76%, respectively (22–24). Moreover, effective thrombolytic therapy requires proper catheter placement, long treatment duration, and the need for surgical reconstruction or endovascular procedures after thrombolysis (10,22). Adjunctive therapies that accelerate thrombolysis and reduce thrombolytic agent dose are desirable. Mechanical thrombectomy increases lysis with a reduction of systemic complications and thrombolytic agents (10,25,26); however, these tools require intraluminal passage through the occlusion and are more suitable in the treatment of acute thrombus (10,26). Some have limitations on vessel size and can be prone to kinking when a crossover approach is used (26). Moreover, dissections, perforations, and residual thrombus have been reported in as many as 30% of cases with some of the systems (10,26). Finally, most clinical studies pertaining to mechanical thrombectomy devices are retrospective (10,26).

US offers the advantages of accelerating reperfusion without increasing systemic bleeding complications (8). The proposed mechanisms of US-enhanced thrombolysis are multiple, complex, and dependent on the acoustic parameters used (7,8). Although commercial catheter-delivered intravascular low-intensity US has shown some improvement, it requires vessel catheterization and continuous delivery of a low-intensity beam over a period of several hours (11,13). More importantly, randomized trials comparing intravascular low-intensity US to standard pharmacologic thrombolysis are currently under way (12) but not yet published (10). In the present study, focused high-intensity pulsed application was delivered transcutaneously. The vessel was catheterized for angiograms and TPA administration but not pHIFU treatments. It is conceivable that, in a clinical setting, pHIFU can be delivered in the recovery area followed by transfer of the patient to the interventional radiology suite for thrombolytic agent administration and remaining intervention.

As in previous publications in small animals, pHIFU alone did not result in significant thrombolysis, as evident on the angiograms obtained after sham pHIFU and before TPA administration (18,19). These angiograms were obtained immediately after sham pHIFU, and it would be unlikely that additional effects of pHIFU without TPA would have appeared if further time had elapsed, as the bypass graft model is designed to reocclude within 60 minutes unless the thrombus is eliminated and the stenosis at the distal anastomosis is treated. Therefore, pHIFU alone did not have an angiographically detectable clot lysis effect,

which would have raised concerns for large emboli. Previous in vitro and small-animal in vivo studies have shown that pHIFU enhanced pharmacologic thrombolysis (7,18,19). This effect is not limited to TPA; Luo et al (27) observed an average 25% increase of thrombolysis when combining pulsed US exposures of 2.2 W/cm² and streptokinase or urokinase in an in vitro model. The exact mechanisms of pHIFU augmentation of thrombolysis are poorly defined, but probably include mechanical, shear, or radiation forces. It has been shown that pHIFU increases the depth of penetration of plasminogen activators within the thrombus in addition to altering their distribution and localization in the clot (7,28,29). In a recent publication, Jones et al (28) described increased exposed fibrin on scanning electron microscopic examination of thrombus treated with pHIFU as opposed to controls. The authors also demonstrated, by fluorescence recovery after photobleaching, that pHIFU significantly increased the diffusion coefficient of dextrans with a molecular weight similar to that of TPA (70 kDA) (28). Although the spatial-peak intensity during the pulse at the focus was 857 W/cm², insignificant heat is generated such that thermal effects are not likely influential. Indeed, direct temperature measurements with similar exposures and higher intensities than in our study for drug delivery resulted in temperature elevation of a few degrees Celsius (30,31). The temperature was not monitored during the actual treatments because of concerns that the thermometry probe might interfere with the US waves and alter the results if placed within or near the focal zone or yield misleading measurements if placed outside of the beam. However, the temporal-average intensity at the focal zone was low, which does not warrant concern for significant tissue heating. Moreover, histopathologic examination of the specimens did not reveal any evidence of coagulative necrosis. In the absence of a thermal mechanism for enabling improved bioavailability of TPA leading to enhanced thrombolysis, cavitation or mechanical effects could have been produced. The latter is a recently proposed mechanism described by Hancock et al (32), which involves generation of shear forces produced by radiation force–induced displacements and results in the creation of structural defects (gaps or conduits in the tissue) enabling improved penetration of agents, as seen by Jones et al (28).

To forgo the use of thrombolytic agents completely, pHIFU and microbubbles (without any thrombolytic agents) reportedly have been successful in reestablishing patency, although their use is sometimes associated with soft-tissue injuries or skin necrosis (11,16,17). Moreover, this approach may have regulatory hurdles that could impede translation into the clinic.

The present study's primary objective was to determine if the addition of pHIFU to pharmacologic thrombolytic agents increased thrombolysis of a 7-day-old thrombus in a large animal model. Most preclinical published literature (6,7,11,14,15) examines reperfusion of acute thrombus (<12 h). However, thrombus composition and response to thrombolytic agents change with time (23,33,34). The present study's model had the advantage of reproducing the clinical scenario with respect to graft material, physiology of graft failure, thrombus burden, and maturation. To eliminate any confounding factors, the control group was carefully treated with sham pHIFU such that the only difference was the absence of sonication energy. The artifact produced by sonication on the US screen prevented the interventions from being blinded. The image analysis and histopathologic analysis were performed by a blinded reviewer and a board-certified independent blinded pathologist, respectively. Measuring percent thrombolysis by CT was considered; however, a CT angiogram with peripheral contrast medium administration might not fully opacify patent graft areas compared with a local injection. CT with local contrast agent injection is associated with considerable beam-hardening artifacts as a result of high concentration of contrast agent within the lysis catheter and unnecessary radiation exposure to the operator. Therefore, the increase of patent graft area was examined by angiography, consistent with clinical practice and previous publications (19,35,36). The use of an edge detection program

increased the accuracy and reproducibility of the contrast area measurements as opposed to manual segmentation; adjustments were made by the blinded reviewer if necessary. The angiograms were negative images, meaning that contrast had lower pixel value. There was no statistical difference between treatment groups in average pixel intensity within the areas of graft opacified by contrast medium on baseline and 30-minute angiograms ($P = .33$ and $P = .86$, respectively). A difference would have suggested that the measurement technique was inconsistent or that the thrombolysis induced by each technique is different. For example, if the pixel intensity values were significantly higher in one group, it could mean that the measured contrast area in that group included nonrecanalized areas. Regardless of the patent graft area on the baseline angiogram, pHIFU and TPA led to an increase of recanalized graft area by 51.8%, compared with 9.1% in the sham pHIFU/TPA group, with narrow SDs. One of the sham-treated animals appeared to show greater patency at baseline compared with the final angiogram. This might be explained by the fact that additional manipulations were needed to catheterize the graft, potentially leading to the breakup of thrombus. The graft reoccluded during sham pHIFU, and there was some recanalization after TPA administration, of note, in all other animals, the graft was catheterized on the first attempt.

The present study's limitations include a small number of animals; however, a significant difference with a small SD was observed at the interim analysis despite the penalty for early stoppage. Conducting further experiments would have been inconsistent with the principles of reduction in animal use in biomedical research. Second, temperature measurements were not performed during experiments because of concerns that the thermometry probe would interfere with the beam. Although pHIFU alone did not produce angiographically detectable thrombolysis, evaluation for emboli after pHIFU and TPA administration was not performed because the grafts were not fully revascularized at the end of our experiments. Finally, percentage of thrombolysis was estimated on angiography, consistent with clinical practice; however, explanting the grafts and performing direct thrombus measurements would have been a stronger methodology. This was not performed because explanting the grafts for direct thrombus measurement would compromise histopathologic analysis of the tissue and grafts.

In conclusion, pHIFU increased thrombolysis as quantified by angiography in a statistically and clinically significant manner, without any differences on histopathologic analysis compared with sham pHIFU in a 7-day-old thrombus model.

Acknowledgments

This work was supported in part by the National Institutes of Health (NIH) Intramural Research Program Grant 1Z01CL046011-01. This project is a collaboration between the NIH and the Food and Drug Administration as part of an Interagency Agreement.

The authors acknowledge Hilary Hancock, BSc, and Ali Ziadloo, BE, for their contributions.

Appendix: Pulsed High-Intensity-Focused Ultrasound Device and Exposures

The spherically focused pulsed high-intensity-focused ultrasound (HIFU) transducer has a focal length (ie, radius of curvature) of 10 cm. A custom water bolus apparatus was fabricated (CIVCO Medical Solutions, Kalona, Iowa) to allow adjustments of the distance between the transducer and the skin, in order to move the fixed focal zone to the appropriate skin to target depth. The transducer is driven by a pulse-wave generator incorporated with a linear amplifier. Electrical power was monitored by a real-time peak pulse power meter (Angiosonics, Morrisville, North Carolina). A US imaging transducer (C8-5 8- to 5-MHz

broadband curved array; Philips, Bothell, Washington) was integrated in the center of the HIFU transducer to provide real-time guidance for positioning and monitoring.

The acoustic output of the therapeutic transducer was measured by using a radiation force balance system in which an absorbing brush target is suspended in a water bath from an electronic balance (HM-202; AND, Bradford, Massachusetts) with the transducer positioned to radiate downward on the target. The device controls that affect the radiated US energy are the indicated power (electrical), HIFU cycle-on time, HIFU cycle-off time, and number of cycles per site. The operating conditions during the in vivo experiments comprised an indicated power setting of 90 W, 100-ms cycle-on time, 900-ms cycle-off time, and 60 cycles per site. This setting corresponded to a 10% duty cycle. The free-field measured acoustic power during the pulse is 71.3 W, corresponding to a transducer efficiency of 80%.

The focal field of the therapeutic transducer was characterized with a calibrated hydrophone (Onda, Sunnyvale, California). To protect the hydrophone, a low power of 4 W was used. From these measurements, the -6 dB focal dimensions, based on the root mean square pressure, are $f_x = f_y = 1.4$ mm and $f_z = 21$ mm, where x and y represent orthogonal lateral dimensions and z is the axial dimension (ie, in the direction of US propagation). To compare these measurements with simulation results, the Khokhlov/Zabolotskaya/Kuznetsov-based propagation model was used at a low power. The calculated -6 dB focal dimensions from the peak negative pressure waveform were $f_x = f_y = 1.95$ mm and $f_z = 17.7$ mm. The -6 dB focal dimensions from the peak positive pressure waveform were $f_x = f_y = 1.65$ mm and $f_z = 16.7$ mm, results consistent with the hydrophone results given the measurement uncertainty.

To obtain a more accurate assessment of the actual in situ acoustic pressure at the focus within the tissue, simulations from a two-layer nonlinear propagation model based on the Khokhlov/Zabolotskaya/Kuznetsov equation were performed by using the measured acoustic power for the device transducer (1). The total attenuation coefficient and speed of sound were estimated based on attenuation and sound speed values for skin, fat, and muscle reported in the literature (2). The simulation yielded the axial pressure, intensity waveforms, axial harmonic distribution, pressure waveform on the axis at the location of peak positive pressure, and focal values for the peak positive and negative pressures and intensity. The values of the peak positive and peak negative pressures at the focus were 7.4 MPa and 4.2 MPa, respectively. The spatial-peak intensity during the pulse at the focus was 857 W/cm².

For the in vivo experiments, the imaging transducer was rigidly fixed in the coaxial opening and the water bolus applied to the HIFU transducer. The location of the focal zone relative to the imaging transducer was confirmed by sonicating an agarose gel phantom-containing albumin by using 100% duty cycle for 5 seconds at a device setting of 90 W. The ablation zone was characterized by increased echogenicity of the gel phantom as a result of coagulation of the albumin.

Bypass Graft Material

The bypass graft was created with a straight 6-mm-diameter gelatin-sealed reinforced expanded polytetrafluoroethylene graft (Vascutek, Terumo, Ann Arbor, Michigan). These grafts were chosen in view of their widespread clinical use (3).

Bypass Graft Model Development

In the initial animals, a proximal end-to-side anastomosis was used, but was replaced by an end-to-end anastomosis because it simplified subsequent catheterization of the bypass graft. In addition, a single long incision was used initially during model development and replaced

by two smaller separate incisions for the proximal and distal anastomosis with the graft tunneled deep to the sternocleidomastoid muscle.

The animals were examined by a veterinarian at 12, 24, 48, and 72 hours after surgery, with particular attention paid to signs of infection, pain, and/or discomfort as demonstrated by changes in diet habits, normal behavior, and ambulation.

Image Analysis

In the dose response experiments, the area of contrast within the graft was traced on the angiograms at each time point for each dose. The area of the contrast measured (in pixels) at a given time point for each dose was divided by the area measured on the pretreatment angiogram to get a percentage measurement of thrombolysis.

For the sham pulsed HIFU animals, the limits of the graft were marked by two clamps, using the computed tomography table positions at the proximal and distal ends of the graft. The graft length was measured on the angiogram along the course of the thrombolysis catheter. The 5-cm distance between the two radiopaque markers of the lysis catheter was used to correct for magnification and provide the graft width (6 mm) in the image space (normalized for magnification). The graft dimensions (width and length) were used to calculate the graft area in the image space.

Pathologic Analysis

The specimens were bisected parallel to the skin surface to produce two blocks (skin surface and graft surface), and each tissue block was sectioned at 1-cm intervals. The trimmed tissues were processed for paraffin embedding and each block was sectioned in two and stained with hematoxylin and eosin and Movat pentachrome. Pathologic analysis of the specimens was performed by a board-certified blinded pathologist at an outside independent institution. The overall integrity of the intervening tissues and graft were assessed. Histopathologic findings were scored by the following criteria: grade 1 indicated minimal change, whereby change barely exceeded normal limits; grade 2 indicated mild change, defined as the lesion being easily visible but of limited severity and extent; grade 3 indicated moderate change, whereby there was a prominent lesion that did not affect the majority of tissue; and grade 4 indicated severe change, defined as a marked change affecting the majority of the tissue.

References

1. Soneson JE, Myers MR. Gaussian representation of high-intensity focused ultrasound beams. *J Acoust Soc Am* 2007; 122:2526–2531.
2. Collins DE. ICRU Report 61: tissue substitutes, phantoms and computation modelling in medical ultrasound. Albuquerque, NM: American Society of Radiologic Technologists, 1998.
3. Chakfe N, Dieval F, Thaveau F, et al. Vascular graft prosthesis. *Ann Chirurg* 2004; 129:301–309.

References

1. Fatourou EM, Paraskevas KI, Seifalian AM, Hamilton G, Mikhailidis DP. The role of established and emerging risk factors in peripheral vascular graft occlusion. *Expert Opin Pharmacother*. 2007; 8:901–911. [PubMed: 17472536]

2. Kapadia MR, Popowich DA, Kibbe MR. Modified prosthetic vascular conduits. *Circulation*. 2008; 117:1873–1882. [PubMed: 18391121]
3. Dormandy JA, Rutherford RB. Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC). *J Vasc Surg*. 2000; 31(suppl):S1–S296. [PubMed: 10666287]
4. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *Eur J Vasc Endovasc Surg*. 2007; 33(suppl 1):S1–75. [PubMed: 17140820]
5. Motarjeme A. Ultrasound-enhanced thrombolysis. *J Endovasc Ther*. 2007; 14:251–256. [PubMed: 17484537]
6. Rosenschein U, Furman V, Kerner E, Fabian I, Bernheim J, Eshel Y. Ultrasound imaging-guided noninvasive ultrasound thrombolysis: preclinical results. *Circulation*. 2000; 102:238–245. [PubMed: 10889137]
7. Pfaffenberger S, Devic-Kuhar B, El-Rabadi K, et al. 2MHz ultrasound enhances t-PA-mediated thrombolysis: comparison of continuous versus pulsed ultrasound and standing versus travelling acoustic waves. *Thromb Haemost*. 2003; 89:583–589. [PubMed: 12624644]
8. Francis CW. Ultrasound-enhanced thrombolysis. *Echocardiography*. 2001; 18:239–246. [PubMed: 11322907]
9. Blinc A, Francis CW, Trudnowski JL, Carstensen EL. Characterization of ultrasound-potentiated fibrinolysis in vitro. *Blood*. 1993; 81:2636–2643. [PubMed: 8490172]
10. Lyden SP. Endovascular treatment of acute limb ischemia: review of current plasminogen activators and mechanical thrombectomy devices. *Perspect Vasc Surg Endovasc Ther*. 2010; 22:219–222. [PubMed: 21411460]
11. Siegel RJ, Luo H. Ultrasound thrombolysis. *Ultrasonics*. 2008; 48:312–320. [PubMed: 18462769]
12. Schrijver AM, Reijnen MM, van Oostayen JA, et al. Dutch randomized trial comparing standard catheter-directed thrombolysis versus ultrasound-accelerated thrombolysis for thromboembolic infrainguinal disease (DUET): design and rationale. *Trials*. 2011; 12:20. [PubMed: 21255459]
13. Schrijver A, Vos J, Hoksbergen AW, et al. Ultrasound-accelerated thrombolysis for lower extremity ischemia: multicenter experience and literature review. *J Cardiovasc Surg*. 2011; 52:467–476. [PubMed: 21792154]
14. Culp WC, Erdem E, Roberson PK, Husain MM. Microbubble potentiated ultrasound as a method of stroke therapy in a pig model: preliminary findings. *J Vasc Interv Radiol*. 2003; 14:1433–1436. [PubMed: 14605109]
15. Culp WC, Porter TR, Lowery J, Xie F, Roberson PK, Marky L. Intracranial clot lysis with intravenous microbubbles and transcranial ultrasound in swine. *Stroke*. 2004; 35:2407–2411. [PubMed: 15322299]
16. Culp WC, Porter TR, McCowan TC, et al. Microbubble-augmented ultrasound declotting of thrombosed arteriovenous dialysis grafts in dogs. *J Vasc Interv Radiol*. 2003; 14:343–347. [PubMed: 12631639]
17. Nishioka T, Luo H, Fishbein MC, et al. Dissolution of thrombotic arterial occlusion by high intensity, low frequency ultrasound and dodecafluoro-pentane emulsion: an in vitro and in vivo study. *J Am Coll Cardiol*. 1997; 30:561–568. [PubMed: 9247533]
18. Frenkel V, Oberoi J, Stone MJ, et al. Pulsed high-intensity focused ultrasound enhances thrombolysis in an in vitro model. *Radiology*. 2006; 239:86–93. [PubMed: 16493016]
19. Stone MJ, Frenkel V, Dromi S, et al. Pulsed-high intensity focused ultrasound enhanced tPA mediated thrombolysis in a novel in vivo clot model, a pilot study. *Thromb Res*. 2007; 121:193–202. [PubMed: 17481699]
20. Mortensen, EN.; Barrett, WA. Proceedings of SIGGRAPH 1995. Vol. 95. New York: Association for Computing Machinery; 1995. Intelligent scissors for image composition; p. 191–198.
21. Shun Z, Yuan W, Brady WE, Hsu H. Type I error in sample size re-estimations based on observed treatment difference. *Stat Med*. 2001; 20:497–513. [PubMed: 11223896]
22. Huettl EA, Soulen MC. Thrombolysis of lower extremity embolic occlusions: a study of the results of the STAR Registry. *Radiology*. 1995; 197:141–145. [PubMed: 7568812]

23. Hess H, Mietaschk A, Bruckl R. Peripheral arterial occlusions: a 6-year experience with local low-dose thrombolytic therapy. *Radiology*. 1987; 163:753–758. [PubMed: 3575727]
24. McNamara TO, Bomberger RA, Merchant RF. Intra-arterial urokinase as the initial therapy for acutely ischemic lower limbs. *Circulation*. 1991; 83:1106–1119. [PubMed: 1991392]
25. Comerota AJ. Development of catheter-directed intrathrombus thrombolysis with plasmin for the treatment of acute lower extremity arterial occlusion. *Thromb Res*. 2008; 122(suppl 3):S20–S26. [PubMed: 18701142]
26. Wissgott C, Kamusella P, Andresen R. Percutaneous mechanical thrombectomy: advantages and limitations. *J Cardiovasc Surg*. 2011; 52:477–484. [PubMed: 21792155]
27. Luo H, Steffen W, Cercek B, Arunasalam S, Maurer G, Siegel RJ. Enhancement of thrombolysis by external ultrasound. *Am Heart J*. 1993; 125:1564–1649. [PubMed: 8498294]
28. Jones G, Hunter F, Hancock HA, et al. In vitro investigations into enhancement of tPA bioavailability in whole blood clots using pulsed-high intensity focused ultrasound exposures. *IEEE Trans Biomed Eng*. 2010; 57:33–36. [PubMed: 20064753]
29. Francis CW, Blinc A, Lee S, Cox C. Ultrasound accelerates transport of recombinant tissue plasminogen activator into clots. *Ultrasound Med Biol*. 1995; 21:419–424. [PubMed: 7645133]
30. Frenkel V, Etherington A, Greene M, et al. Delivery of liposomal doxo-rubicin (Doxil) in a breast cancer tumor model: investigation of potential enhancement by pulsed-high intensity focused ultrasound exposure. *Acad Radiol*. 2006; 13:469–479. [PubMed: 16554227]
31. Patel PR, Luk A, Durrani A, et al. In vitro and in vivo evaluations of increased effective beam width for heat deposition using a split focus high intensity ultrasound (HIFU) transducer. *Int J Hyperthermia*. 2008; 24:537–549. [PubMed: 18608578]
32. Hancock HA, Smith LH, Cuesta J, et al. Investigations into pulsed high-intensity focused ultrasound-enhanced delivery: preliminary evidence for a novel mechanism. *Ultrasound Med Biol*. 2009; 35:1722–1736. [PubMed: 19616368]
33. Wholey MH, Maynar MA, Wholey MH, et al. Comparison of thrombo-lytic therapy of lower-extremity acute, subacute, and chronic arterial occlusions. *Cathet Cardiovasc Diagn*. 1998; 44:159–169. [PubMed: 9637438]
34. Weaver FA, Comerota AJ, Youngblood M, Froehlich J, Hosking JD, Papanicolaou G. Surgical revascularization versus thrombolysis for nonembolic lower extremity native artery occlusions: results of a prospective randomized trial. The STILE Investigators. *Surgery versus Thrombolysis for Ischemia of the Lower Extremity*. *J Vasc Surg*. 1996; 24:513–521. [PubMed: 8911400]
35. Lin PH, Chen C, Surowiec SM, et al. A porcine model of carotid artery thrombosis for thrombolytic therapy and angioplasty: application of PTFE graft-induced stenosis. *J Endovasc Ther*. 2000; 7:227–235. [PubMed: 10883961]
36. Lin PH, Surowiec SM, Conklin B, et al. An endovascular model of carotid stenosis for the evaluation of thrombolysis and angioplasty. *J Endovasc Ther*. 2000; 7:486–493. [PubMed: 11194820]

Abbreviations

HIFU	high-intensity focused ultrasound
pHIFU	pulsed high-intensity-focused ultrasound
TPA	tissue plasminogen activator

Figure 1. Multiplanar reformatted reconstruction of CT angiography images of the thrombosed graft demonstrate a 40% diameter stenosis near the distal anastomosis, which translates to 50%–60% area reduction of the lumen (measured on CT axial images; not shown).

Figure 2.

Pretreatment and 30-minute posttreatment angiograms in the sham pHIFU group (upper row) and pHIFU group (lower row). The clamps delineate the extremities of the graft per CT imaging. The infusion segment of the lysis catheter (between the two radiopaque markers) is positioned within the graft. A pretreatment angiogram confirms graft thrombosis. Not much improvement is seen in the sham group (top row), as opposed to a 50% recanalization of the graft in the pHIFU group (bottom row). Also shown is the Livewire tracing of the contrast area on the 30-minute angiogram.

Figure 3.

Percentage of thrombolysis. The graft dimensions (width and length) were used to calculate the graft area in image space (adjusted for magnification). The area of the graft opacified by contrast agent was traced on the angiograms at each time point. That area was divided by the graft area to give the percentage of patent graft area at each time point. The difference in percentage of patent graft area at each time point compared with the pretreatment angiogram represents the percentage of thrombolysis and is demonstrated with the SD at each time point.

Figure 4.

Hematoxylin and eosin-stained pathologic sections containing graft from an animal treated with pHIFU and TPA (**a**) or sham pHIFU and TPA (**b**). The slides demonstrate the grafts surrounded by reactive fibrovascular tissue and fluid accumulation most likely related to postoperative changes. Similar findings are seen in both slides. No additional findings are seen in the pHIFU-treated animal. (Original magnification, $\times 5$.)

Figure 5. Hematoxylin and eosin–stained pathologic section from animals treated with pHIFU and TPA (**a**) or sham pHIFU and TPA (**b**). The slides demonstrate that the graft walls are infiltrated with erythrocytes and fluid. This was similar in both groups in all specimens examined. (Original magnification, $\times 20$.)

Table
Percentage of Thrombolysis Based on Angiograms at Each Time Point per Group

Interval	Sham pHIFU/TPA	pHIFU/TPA	Adjusted <i>P</i> Value
After sham pHIFU, before TPA	−7.38 ± 16.09	23.41 ± 9.00	.0674
10 min after TPA	−3.74 ± 17.94	39.96 ± 4.90	.0133
15 min after TPA	5.93 ± 16.58	46.77 ± 9.16	.0166
30 min after TPA	9.03 ± 13.63	51.79 ± 3.53	.0032
Average intensity of pixel value	14.09 ± 9.16	13.87 ± 4.98	.9664

Note.—Values are presented as means ± SD. A difference between the two groups could have suggested inconsistent measurement technique or different thrombolysis depending on technique, but there was no statistical difference between the two groups in that regard. pHIFU = pulsed high-intensity-focused ultrasound, TPA = tissue plasminogen activator.