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Pulsed-high intensity focused ultrasound enhanced tPA mediated thrombolysis in a novel *in vivo* clot model, a pilot study

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Abstract

Introduction—Thrombotic disease continues to account for significant morbidity and mortality. Ultrasound energy has been investigated as a potential primary and adjunctive treatment for thrombotic disease. We have previously shown that pulsed-high intensity focused ultrasound (HIFU) enhances thrombolysis induced by tissue plasminogen activator (tPA) *in vitro*, including describing the non-destructive mechanism by which tPA availability and consequent activity is increased. In this study we aimed to determine if the same effects could be achieved *in vivo*.

Materials and Methods—In this study, pulsed-HIFU exposures combined with tPA boluses was compared to treatment with tPA alone, HIFU alone and control in a novel *in vivo* clot model. Clots were formed in the rabbit marginal ear vein and verified using venography and infrared imaging. The efficacy of thrombolytic treatment was monitored via high resolution ultrasonography for five hours post treatment. The cross-sectional area of clots at 4 points along the vein was measured and normalized to the pre-treatment size.

Results—At five hours the complete recanalization of clots treated with pulsed-HIFU and tPA was significantly different from the partial recanalization seen with tPA treatment alone. tPA treatment alone showed a significant decrease in clot versus control, where HIFU was not significantly different than control. Histological analysis of the vessel walls in the treated veins showed no apparent irreversible damage to endothelial cells or extravascular tissue.

Conclusions—This study demonstrates that tPA mediated thrombolysis can be significantly enhanced when combined with non-invasive pulsed-HIFU exposures.

Keywords

High intensity focused ultrasound (HIFU); thrombolysis; tissue plasminogen activator (tPA); clot model; rabbit ear vein

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Thrombotic disease continues to be a major cause of morbidity and mortality in multiple forms, including stroke and myocardial infarction, as well as venous thromboembolism. Ultrasound has been investigated as a means to induce or enhance thrombolysis [1-3]. However, the ultrasound technology employed for directly inducing thrombolysis has involved procedures that are either invasive (e.g. via catheter based techniques) [3-6] or rely on ultrasound mechanisms such as acoustic cavitation [7], which is potentially damaging to surrounding tissues [8]. Ultrasound has also been combined with thrombolytic agents in *in vitro* and *in vivo* models with improved rates of thrombolysis [1].

High intensity focused ultrasound (HIFU) is being used today primarily to thermally ablate tissue, where the rate of energy deposition in the tissues produces heat faster than it can be removed. As a result, temperature elevations in the exposed tissue can be reached for local ablation [9]. Pulsed-HIFU exposures, however, using low duty cycles (i.e. the relative time 'ON' during each pulse), generate energy deposition rates low enough that temperature elevations are well below the threshold for thermal damage in the tissues [10]. The effects of pulsed-HIFU are more mechanical in nature, such as the creation of acoustic radiation forces. These forces, if high enough, can produce local displacements on the order of cellular dimensions [11,12]. It has been suggested that repetitive displacements are capable of inducing structural alterations in the tissue, through the creation of locally induced strain. The strain results from shear forces produced between adjacent regions of tissue experiencing non-uniform displacement, and the alterations (i.e. widening of intercellular spaces between both endothelial and parenchymal cells) may increase the tissue's permeability [13].

We have applied pulsed-HIFU exposures to non-invasively enhance the delivery of a wide variety of substances in different animal and tissue models, including soluble compounds, plasmid DNA, and nanoparticles, in a reversible and non-destructive manner [14-16]. Pulsed-HIFU also demonstrated the ability to enhance tissue plasminogen activator (tPA)-mediated thrombolysis *in vitro*. The lack of effects, without drug, and elevated D-dimer levels with pulsed-HIFU and drug compared to drug alone, suggest that increased thrombolysis was due to improved activity of tPA into the clots, and not mechanical degradation [17]. This hypothesis is supported by our recent study showing that pulsed-HIFU produced non-destructive, macroscopic alterations in clots that increased their exposed surface area, and consequently improved binding and penetration of the tPA (18). These studies [17,18] were the basis of the current study, which evaluated the potential of using pulsed-HIFU to enhance tPA-mediated thrombolysis non-invasively *in vivo*. A novel clot model in the marginal ear vein of rabbits was developed specifically for this pilot study.

Materials and Methods

Animal Model

All procedures related to animals were performed under a protocol approved by the Institutional Animal Care and Use Committee of the W. G. Magnuson Clinical Center, National Institutes of Health

We developed a novel clot model using the marginal ear vein of rabbits - a superficial vessel - in order to allow the use of high-resolution ultrasound imaging for monitoring the clots' response to treatment. This type of high resolution imaging would not be possible in larger, deeper vessels due to rapid attenuation of the ultrasound beam at this high frequency. A superficial vessel also allowed for fast and non-invasive verification of the presence of clot with infrared imaging. Another advantage of this new model was the ability to form clots without invasive surgical cut-down, as required to expose deeper vessels for other clot models [19,20].

Female New Zealand White rabbits (3.12-4.8 kg, avg 3.67 kg; ages 119-224 days old; Covance, Denver, PA) were used. For all procedures (clot formation, angiography, ultrasound imaging, infrared imaging, pulsed-HIFU treatments, angiocatheter insertion and tPA or saline infusions) the rabbits were gently restrained in the Bunny Snuggle® restraint device (Lomir Biomedical Inc, Quebec, Canada) sedated with xylazine (4.84 mg/kg, Ben Venue Laboratories, Bedford, OH) and ketamine (28.6 mg/kg; 100 mg/mL, Fort Dodge Animal Health, Fort Dodge, IA) administered subcutaneously, and then anesthetized with isoflurane (2% of 1 L/min O₂).

The dorsal and ventral sides of both ears were shaved. An indelible marker was used to designate the 3-cm region of vein to be clotted, where marks were set at 1 cm intervals starting at 1 cm proximal to the clot and ending 1 cm distal to it, giving the following positions: -1, 0, 1, 2, 3, and 4. These marks were placed perpendicular to the long axis of the vein and served as guides for the ultrasound probe. Pre-clot ultrasound images (Visualsonics, Toronto, Ontario) were taken at all 6 marks along the vein and saved as freeze frame and cine loop images, as described in the ultrasound imaging section (below).

Prior to clot formation the ears were swabbed with sterile alcohol in the region where the clots would be formed. Aortic cross clamps (Morris Aorta Clamp, 8", 37-1700 Codman, Raynham, MA) were clamped onto the ear such that they crossed the marginal ear vein at the 0 cm and 3 cm marks, and met medially to exclude communicating veins from the main marginal ear vein (Fig. 1A). 0.04 ml of bovine thrombin (10,000 U/mL in saline; GenTrac, Inc, Middleton, WI) was then injected into the proximal end of the isolated marginal ear vein using a 30-G needle (Becton Dickinson and Co., Franklin Lakes, NJ). This was done by first withdrawing blood into the syringe and then quickly injecting the blood/thrombin mixture into the vein, similar to a procedure described by Agnelli et al. [21]. Slight pressure was then held at the site of injection to prevent bleeding. The clamps were left in place, and the animal was monitored for 20 minutes to allow for clot formation.

Angiography

In order to verify the formation of a viable clot, angiograms were performed 1 hour following the thrombin injection. A 25-G butterfly angiocatheter was inserted into the central ear artery and a 3 mL bolus of contrast material (Isovue 300, Bracco Diagnostics Inc; Princeton, NJ) was injected into the artery. Fluoroscopic images were captured at various phases. This procedure was carried out for 2 animals in preliminary trials, after which it was discontinued when it was determined that a successful procedure for clot formation had been established.

Infrared Imaging

A ThermoCAM™ S60 infrared camera (FLIR Systems, Portland OR) was used for thermographic monitoring of temperature in the rabbit ears, both before and after clot formation. The ThermoCAM™ S60 has a thermal sensitivity of 0.08°C and an accuracy of $\pm 2\%$ of the reading. The camera, secured on a tripod, was angled downward toward the ears. For each image, the camera recording speed was set at 3Hz (3 frames captured per second).

Ultrasound Imaging

In order to image the veins in which the clots were formed, ears were placed on a gauze roll (10 cm $\times$ 4 cm) with the dorsal side of the ear facing up towards the ultrasound probe, and taped to the imaging table (Vibraplane, Kinetic Systems Inc, Boston, MA) using transpore tape (3M, St. Paul, MN). Pre-warmed ultrasound coupling gel (Aquasonic, Parker Laboratories Inc, Fairfield, NJ) was swabbed liberally onto the ear vein. A 55 MHz ultrasound probe (Vevo 660 High Resolution Imaging System, Visualsonics, Toronto, Ontario) attached to a stereotactic stand was lowered and positioned perpendicularly to the long-axis of the vein to capture cross-sectional images. The probe was moved along the vein using the stereotactic stand

(Visualsonics, Toronto, Ontario) to the marks at 1 cm intervals. Freeze frame images and cine loops (30 seconds in length) were taken at each of the six marks. Images were captured pre-clot, 1 hour post-clot (immediately pre-treatment) and at 1, 2, 3 and 5 hours following treatment.

HIFU system

A custom modified Sonoblate 500 (Focus Surgery; Indianapolis, IN) was used to provide all pulsed-HIFU exposures. The probe possessed both a therapeutic (1 MHz) transducer and a co-linear (10 MHz) imaging transducer, each with a focal length of 4 cm. The therapeutic transducer was concave and spherical, with a diameter of 5 cm; the aperture of the planar imaging transducer was 0.8 cm. The maximum power available for the therapeutic transducer was 120 W, which possessed a focusing factor of approximately 1300. The focal zone produced by the therapeutic transducer was ellipsoid in shape, with a radial diameter of 1.38 mm and an axial length of 7.2 mm.

In order to expose the clots, the following set up was used (Fig. 2A). The probe was positioned horizontally with the transducer facing upwards. The transducer was encased in a plexiglass box (15.5 cm × 22.5 cm × 14.5 cm) filled with degassed water. The upper surface of the box had an acoustic permeable membrane (15 cm × 11.5 cm) made of silicone, 0.013 cm thick (McMaster Carr, Atlanta, GA). For referencing, a highly echogenic guide made of magnetic tape (6.5 cm × 2.5 cm) was adhered to the membrane at its geometrical center using transpore tape (3M, St. Paul, MN). The guide had its center cut out (0.7 cm × 4 cm).

Pulsed-HIFU exposures

For pulsed-HIFU exposures the rabbit ear was placed on the acoustic permeable membrane so that the clot was positioned directly over the echogenic guide (Fig. 2B). Acoustic coupling gel was placed at the guide, between the ear and the membrane, and the ear was kept in position using transpore tape. Both the sector (horizontal) and linear (vertical) scans were operated to ensure that the ear was at the center of the focal zone (Fig. 2C). The amount of water in the box could be increased or decreased to raise or lower the membrane, respectively (and therefore the ear), as required. Using the HIFU system's graphic user interface, 15 raster points (2 mm apart) were set from one end (proximal) of the clot to the other (distal). Sixty pulses were given at each raster point. A total acoustic power of 40 W was used for each pulse, with a duty cycle of 5% and pulse repetition frequency of 1 Hz. With this treatment regimen, exposures at an individual raster point lasted 1 min., and the entire exposure required a total of 15 min. For control (saline) or tPA only groups without HIFU, the clots were positioned over the transducer, as mentioned above, and a sham exposure of 15 min. was given.

tPA or saline administration

Prior to real or sham pulsed-HIFU exposure, a 22-G angiocatheter (Abbocath-T, Venisystems, Abbott, Ireland) was placed into the marginal ear vein of the contralateral (untreated) ear. tPA in normal saline (1.0 mg/mL) or an equal volume of normal saline alone was injected into the angiocatheter at 5 time points during the sham or real pulsed-HIFU exposures: immediately prior to the start of the exposure, at 4, 8 and 12 minute time points following the start of the exposure, and immediately at the termination of the exposure (Fig. 2C).

In preliminary trials with various tPA (Alteplase, Genentech) doses (0.5 to 2.0 mg/kg) we determined that 1 mg/kg was the optimal bolus dose for each of the 5 injections. With this dose, clots treated just with tPA only partially recanalized.

Histology

Since extensive recanalization had occurred after 5 hours in the veins treated with tPA plus HIFU, a decision was made to sacrifice the animals in all groups at that time. Following ultrasound imaging at the 5-hour post-treatment time point, all rabbits were euthanized and the section of vein that had been originally injected with thrombin was harvested. The vein was sectioned at each of the six ultrasound imaging points creating cross-sections perpendicular to the long-axis of the clot (same plane as the ultrasound images). The sections were fixed and mounted on glass slides and stained with hematoxylin and eosin. The slides were observed using light microscopy (Olympus BX51TF, Melville, NY) at a magnification of 100 $\times$ and 400 $\times$, and representative images captured with a digital camera (Olympus DP70, Melville, NY) and saved in TIFF format using proprietary software provided by the company.

Data Processing and Statistical Analysis

The ultrasound images were used to determine the cross sectional area of the clots. Using MIPAV software (v2.2, NIH, Bethesda, MD) the outer circumference of the clot was traced to determine its area, which was recorded in pixels. The mean area of each clot, at an individual time point, was calculated from the values determined at each of the four marks along that clot (0, 1, 2 & 3 cm). After determining the area of the original clot (i.e. following its formation, prior to treatment), subsequent areas at 1, 2, 3 & 5 hrs. post-treatment were measured and normalized relative to the original area. The clots from each experimental group were then presented as a mean at each of the time points, and plotted according to the manner in which their relative size varied over the study period. A Tukey-Kramer HSD test was carried out, using a JMP (Gary, NC) software package, to determine if significant differences occurred between the experimental group means at each of the time points. A P-value less than 0.05 for differences was considered to be significant.

Results

Novel Clot Model Validation

The clot formation procedure was standardized through preliminary experimentation by varying the thrombin dose and clamping time (data not presented here) to produce a clot that remained for at least 24 hours. Angiography demonstrated the presence of clot one hour after formation. This was indicated by the complete lack of contrast in a previously patent vessel (Fig. 1C & D). Increased flow of contrast observed through the contralateral vein further corroborated the presence of intravascular clot. Decreased surface temperature in the region of clotted marginal ear vein was further indication of blood clot as a function of decreased blood flow to that area as seen in Figure 1E & F. Infrared imaging allowed rapid, real time and non-invasive monitoring of clot formation during the preliminary clot model standardization trials. Additional corroboration of clot formation was obtained through high resolution ultrasound, as seen in a representative cross-sectional image (Fig. 3B).

Evaluating thrombolytic treatment efficacy

A total of 16 rabbits were used for this study, divided into four experimental groups: saline and sham HIFU exposure (n = 3); saline and HIFU exposure (n = 3); tPA and sham HIFU exposure (n = 5); and tPA and HIFU exposure (n = 5). The cross-sectional area of each clot (averaged from measurements at four locations) was measured 1, 2, 3, and 5 hours after treatment and expressed as a fraction of the area at time '0'. After 5 hrs the relative changes in the size of the clots treated with pulsed-HIFU and saline (0.90 $\pm$ 0.05 of the original area) were not significantly different from those treated with saline only (1.11 $\pm$ 0.30). The clots treated with tPA alone, however, were significantly smaller (0.78 $\pm$ 0.27) than the saline-treated controls. Clots treated with the combination of pulsed-HIFU and tPA were significantly

different than those treated with tPA alone, having virtually disappeared after 5 hours (0.04 ± 0.06 of the original area). The ear vein of one of the rabbits from the HIFU and tPA was completely recanalized at 2 hrs post-treatment; the vein in a second and third rabbit was similarly recanalized at 3 and 5 hrs, respectively. At 5 hrs, the largest clot in this group was 11% of its original size; less than one quarter the relative size of the smallest clot in the tPA only group at the same timepoint. Dynamic changes in relative clot size for all groups are summarized in Table 1 and Figure 3.

Histology

Histological sections were prepared from the veins of all 16 experimental animals in the study after the final imaging time point (5 hrs.). Observations with light microscopy in both sham and HIFU treated vessels, revealed signs of mild edema and inflammation, including some extravascular neutrophils and focal red cell extravasation. However, no evidence was found of endothelial breaks or disruption in any of the vessels of either group. Representative images of the vessel wall in a vein of each group appear in Figure 4.

Discussion

Pulsed-HIFU has previously been used to enhance the delivery of various substances in animal tumor models, including chemotherapeutic agents, nanoparticles, and plasmid DNA [13], and was shown to increase tPA-mediated thrombolysis *in vitro* [17]. In this study we demonstrated that pulsed-HIFU can also significantly enhance tPA-mediated thrombolysis in a novel *in vivo* model. Whereas rabbit ear-vein clots treated with tPA alone only partially recanalized by 5 hours, complete recanalization was observed when tPA was administered in combination with pulsed-HIFU.

tPA binds to fibrin, thus limiting the extent to which it can diffuse into clots, and reducing its thrombolytic effect [22]. Our recent *in vitro* work, using scanning electron microscopy, has shown that pulsed-HIFU creates channels within clots, providing new routes for transport into their interior. Using fluorescently labeled antibodies to tPA, we showed how these non-destructive changes enabled improved penetration and binding of the drug, both at the clots' surface and within its interior [18]. This mechanism would seem to explain the enhancing effect of pulsed-HIFU on tPA-mediated thrombolysis observed in our *in vitro* [17] study, and similarly could explain the results in the present study. These results are supported by additional studies in purified fibrin gels, where ultrasound exposures caused reversible fiber disaggregation [23], and consequently improved fluid flow through the gels [24]. When the same exposures were carried out in blood clots, improved penetration of tPA was observed, in comparison to untreated clots [25].

Other forms of ultrasound energy have previously been used to promote thrombolysis. Extracorporeal ultrasound has been combined with thrombolytic drugs in clinical trials to treat both acute myocardial infarction [26] and acute middle cerebral artery stroke [27] with promising results. However, both of these treatment methods require further validation and optimisation. In these high-impact studies, long exposures (1 hour or greater) were used with relatively low intensity ultrasound. Ultrasound microbubble contrast agents have been also been combined with intravenous tPA in a clinical trial to achieve significant increases in vessel recanalisation [28]. The use of these agents, however, enhances the activity of acoustic cavitation, which can damage tissue and so might limit the clinical application of this technique [8]. Ultrasound energy delivered from an external ultrasound source through a vibrating wire probe has been investigated *in vitro* [3,4], in canine models [5] and in clinical trials [6] with resultant increased thrombolysis. These methods carry the risks of an invasive endovascular procedure, including vessel rupture, and may not provide substantial benefit over other available invasive endovascular interventions [29]. Ultrasound energy has also been delivered

from catheter-tipped transducers to successfully increase thrombolysis with urokinase *in vitro* [3] and *in vivo* [30]. These catheter based approaches also possess the risks inherent to invasive endovascular procedures.

Ultrasound is capable of generating biological effects from both thermal and non-thermal mechanisms, which may then be used for therapeutic applications [2]. In their comprehensive review on ultrasound thrombolysis, Pfaffenberger et al [1], discussed only non-thermal ultrasound mechanisms for improving thrombolysis with tPA. Employing the same ultrasound exposure parameters used in the present study, Frenkel et al [17] showed that tPA mediated thrombolysis *in vitro* can apparently occur in the absence of generated heat. In their study, the time required to transfer clots from the HIFU exposure tank to incubations in tPA was approximately 5 min. A separate study, however, using similar exposures showed that temperature elevations of up 42 °C required only 30 s or less to return back to baseline [16]. Sakharov et al [31] have shown that elevating the temperature to 43 °C can enhance tPA mediated thrombolysis, where an increase of 30 % was obtained after 90 min. In the present study, boluses of tPA were given throughout the exposures, where the length of an exposure at an individual raster point was 1 min. Interpolating from the data of Sakharov et al [31], where the time dependency on thrombolysis was linear, an increase thrombolysis of only 0.33 % would have occurred during in that one minute period. The absence of a thermal mechanism for enhancing the effects of thrombolytics may also be inferred from the study by Shlamovitz et al [32], where short ultrasound exposures of 10 s were used. In their study, no differences were found whether ultrasound exposures were given before or during incubations in streptokinase. Despite this evidence, however, there is a potential for a thermal mechanism being involved in producing the results presented in this study. Further investigations will be required to ultimately make this determination.

Acoustic cavitation is the most widely regarded ultrasound mechanism for enhancing thrombolysis [1]. Inertial cavitation, which involves the collapse of bubbles and potentially highly destructive effects [8], may perhaps be excluded as a possible mechanism in this study. Damage was not observed in clots *in vitro* using the same exposures [18], nor in the endothelium of vessels in the present *in vivo* study. Furthermore, in neither study was enhanced thrombolysis found to occur as a result of the exposures alone. Stable, or non-inertial, cavitation however has been proposed as a mechanism for enhancing the delivery of drugs without accompanying destructive effects. This includes improving delivery of contrast agents across the blood brain barrier [33], well as thrombolytic agents into blood clots [25]. The manner by which stably oscillating bubbles may alter permeability and improve delivery in biological tissues is, however, complex where a variety of phenomena may occur [34]. Other non-thermal mechanisms, such as radiation forces, have been proposed for both augmenting tPA mediated thrombolysis [1,17], as well as drug delivery in other tissue models [13]. Locally induced displacements by these forces, occurring non-destructively and with minimal generation of heat [11,12], are thought to create structural alterations on a macroscopic scale, rendering the tissue more permeable to therapeutic agents [13]. For example, in one study, Frenkel et al. [17] found that increased rates of tPA mediated thrombolysis correlated well with higher radiation forces and consequent displacements.

The non-destructive nature of the pulsed-HIFU exposures in the present study is supported by the thermal dose of the exposures, which were well below that required to produce coagulative necrosis [35]. The same exposures in other tissue models also showed no destructive effects [15,16]. In the present study, histological analysis of the vessel walls in the treated veins showed no indications of damage in the endothelial cells or extravascular tissue. Similar degrees of mild edema and inflammation were observed in sham and HIFU treated vessels, which can be attributed to handling of the ears. These results are supported by those found by Hwang et al. [36] where 1 MHz pulsed-HIFU exposures were also given in the marginal ear vein of rabbits,

however, at an energy deposition rate over twice that of the present study. Scanning electron microscopy showed that even at these much higher energy levels, there was no damage produced to the endothelium. Moreover, no evidence was found of perivascular collagen degeneration, pyknotic nuclei, and abnormal vacuolization of smooth muscle cells that are indicative of thermal damage. Only when ultrasound contrast agents were added [which lower the pressure threshold for the inception of acoustic (inertial) cavitation, and which consequently may be highly destructive], was any damage observed; and these effects were confined to the luminal surface of the blood vessels. The observations in the present study are, however, still only preliminary. More in-depth investigations are required to confirm that this pulsed-HIFU regimen does not cause tissue damage.

In conclusion, this pilot study demonstrated how thrombolysis can be significantly enhanced *in vivo* when pulsed-HIFU exposures are combined with tPA treatment. With further research in to the mechanisms and safety of the exposures, this technique could potentially be developed for clinical applications, including VTE, arterial thrombosis, and stroke.

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Abbreviations

tPA	tissue plasminogen activator
HIFU	high intensity focused ultrasound

Figure 1.

Clot formation and validation. (A) clamp placement for clot formation in the ear vein of a rabbit; (B) location of clot (short arrows) immediately after clamp removal. (C, D) angiograms of the vasculature in the same rabbit ear: (C) a patent marginal ear vein, five weeks prior to clot formation; (D) a lack of contrast in the marginal ear vein (long arrows) indicating the presence of clot formation, which is further supported by increased contrast flow to the opposite vein. (E, F) images captured with an infrared camera, demonstrating (E) blood flow pre clot and (F) lack of flow post clot in region (short arrows) as depicted by reduced surface temperature (scale to the far right).

Figure 2.

Pulsed-HIFU exposures. (A) the HIFU probe positioned horizontally, with the transducer (lower arrow) in the degassed water tank directed towards the acoustically permeable membrane at the top of the tank (upper arrow). (B) the clotted marginal ear vein (arrows) aligned on the acoustically permeable membrane prior to pulsed-HIFU exposure. (C) screen capture from the collinear imaging transducer used to plan the HIFU exposure, showing a longitudinal view of the rabbit ear at the marginal ear vein. The focal zone is indicated by parallel horizontal lines. The 15 raster points (vertical lines) indicate the location of each 1-minute HIFU exposure point. The temporal sequence (min.) of the five saline or tPA boluses is depicted (arrows), where 1 is the pre-HIFU exposure bolus administration of saline or tPA, and 2, 3, and 4 are given after 4, 8, and 12 min of HIFU, respectively; 5 is given immediately at completion of HIFU.

Figure 3.

High-resolution ultrasound imaging and results of thrombolysis treatment. Upper images: representative high resolution (55 MHz) ultrasound imaging screen captures of the marginal ear vein in cross section: (A) pre-clot, patent vessel; (B) completely clotted vessel, immediately following clot formation procedure; (C) partial recanalisation, post-thrombolytic treatment; (D) more advanced recanalisation post-thrombolytic treatment. The arrows indicate the vein wall in each image. Clot is demonstrated by presence of intravascular hyperechoic structures (B-D). The white bar in all images represents 0.5 mm. Lower graph: Relative size of clots for each of the four treatment groups at 0, 1, 2, 3 and 5 hours post treatment. At 5 hours, significant differences were found between tPA and the saline only groups, and between tPA only and HIFU and tPA.

Figure 4.

Light microscopy observations of vein endothelium. Brightfield images captured of representative sham (A, C) and pulsed-HIFU (B, D) exposed ear veins. A, B: 400 $\times$; C, D 1000 $\times$. No differences were found in the endothelium (arrows) between treated and untreated vessels, which appear intact and undamaged, without the presence of hemorrhage.

Table 1

Relative cross-sectional area of clots in response to therapy

Group	rabbit	Time post-treatment (hrs)			
		0	1	2	3
saline	1	1.00	1.23	1.42	1.43
	2	1.00	0.95	0.86	0.96
	3	1.00	0.90	0.89	0.94
HIFU	1	1.00	0.85	0.85	0.97
	2	1.00	0.91	0.96	0.90
	3	1.00	1.20	0.93	0.80
tPA	1	1.00	0.82	0.69	0.73
	2	1.00	0.84	0.67	0.61
	3	1.00	0.84	0.80	0.84
	4	1.00	0.81	0.75	0.79
	5	1.00	0.64	0.87	0.96
HIFU & tPA	1	1.00	0.59	0.71	0.79
	2	1.00	0.54	0.65	0.57
	3	1.00	0.96	0.00	0.00
	4	1.00	0.48	0.25	0.09
	5	1.00	0.57	0.79	0.53

Each entry is the mean calculated from the values determined from measurements at each of the four locations along that clot (0, 1, 2 & 3 cm).