Ultrasound Imaging–Guided Noninvasive Ultrasound Thrombolysis
Preclinical Results

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Background—Catheter-based therapeutic ultrasound thrombolysis was recently shown to be effective and safe. The purpose of this work was to study the safety and efficacy of external high-intensity focused ultrasound thrombolysis guided by ultrasound imaging in experimental settings.

Methods and Results—A therapeutic transducer was constructed from an acoustic lens and integrated with an ultrasound imaging transducer. In vitro clots were inserted into bovine arterial segments and sonicated under real-time ultrasound imaging guidance in a water tank. With pulsed-wave (PW) ultrasound, the total sonication time correlated with thrombolysis efficiency ($r^2 = 0.7666$). A thrombolysis efficiency of 91% was achieved with optimal PW parameters (1:25 duty cycle, 200-μs pulse length) at an intensity ($I_{spa}$) of $>$35±5 W/cm². Ultrasound imaging during sonication showed the cavitation field as a spherical cloud of echo-dense material. Within <2 minutes, the vessel lumen evidenced neither residual clot nor damage to the arterial wall. On serial filtration, 93±1% of the lysed clot became subcapillary in size (<8 μm). In vitro safety studies, however, showed arterial damage when an $I_{spa}$ of 45 W/cm² was used for periods of ≥300 seconds.

Conclusions—External high-intensity focused ultrasound thrombolysis using optimal PW parameters for periods of ≤300 seconds appears to be a safe and effective method to induce thrombolysis. The procedure can be guided by ultrasound imaging, thereby allowing the monitoring of therapy. (Circulation. 2000;102:238-245.)

Key Words: thrombolysis | ultrasonics

Thrombosis in the cardiovascular system is the leading cause of mortality and morbidity in the western world. The clinical management of vascular thrombosis presents a therapeutic challenge, despite the recent advances in thrombolytic and antiplatelet agents.

Clinical experience with catheter-based therapeutic ultrasound has shown that acoustic energy has the ability to induce effective thrombolysis without injuring the surrounding vessel.1,2 The potential therapeutic use of external high-intensity focused ultrasound (HIFU) was first explored by Lynn et al in 1942.3 They demonstrated that HIFU can induce localized tissue damage at a focal point within the body with no effect on the surface or on the overlying or surrounding tissue. Over the past few years, therapeutic HIFU has been successfully used in neurosurgery, ophthalmology, urology, and oncology.4–7

In an earlier feasibility study, we showed that the use of focused shock waves as a source of acoustic energy provided effective thrombolysis with no damage to the artery.8 However, shock waves are difficult to control, whereas monochromatic ultrasound allows better control of the operating parameters. Thus, the purpose of the present work was to study the safety and efficacy of HIFU thrombolysis guided by ultrasound imaging in experimental settings.

Methods

Therapeutic Ultrasound Transducer

The therapeutic transducer consists of 1–3 piezo-composite material designed as a spherical ring (94-mm diameter, 70-mm radius of curvature). The acoustic lens has a focal point 45 mm from the transducer surface (Angiosonics Ltd). The focal point (~6 dB), measured below the cavitation threshold with a calibrated hydrophone (sprh-s-100, SEA), is tubular (12±1×1.8±0.2×1.8±0.2 mm). The choice of frequency was a balance between the different inherent properties of low- and high-frequency ultrasound. At lower frequencies, ultrasound has a lower cavitation threshold and less attenuation in tissue, but the longer wavelength dictates the need for a larger focal area. At higher frequencies, the cavitation threshold and tissue attenuation are higher, but a smaller focal area can be obtained with shorter wavelength. As a result, the frequency of 500
kHz was used to accommodate the predetermined dimensions of the focal area. The transducer was driven by a pulsed-wave (PW) generator incorporated with a linear amplifier (Angiosonics Ltd). Power was monitored by a real-time peak pulse power meter (Angiosonics Ltd).

An ultrasound imaging transducer (7.5-MHz mechanical annular array) was integrated into the center of the therapeutic transducer in a concentric configuration. Real-time ultrasound imaging allowed identification of the artery and clot and monitoring and control of the cavitation activity and progress of thrombolysis.

In Vitro Studies

Experimental Procedure

Fresh bovine blood (95 mL) was mixed with 7.6% sodium citrate solution (5 mL) and kept at 4°C for up to 3 days. Coagulation was performed by mixing the citrated bovine blood (8 mL) with 1% CaCl2 solution (0.08 mL) in a plastic tube (5.5 mm in diameter). The clots were incubated at 37°C for 2 hours and kept overnight at 4°C. This method yielded a standard clot weight of 333 ± 60 mg (n = 600).

Clots were inserted into fresh bovine carotid artery segment (28 ± 2 mm long with a 7 ± 2-mm diameter). The artery-clot preparations were mounted on a U-shaped frame connected to an X-Y-Z positioning device (ZUK Ltd) and immersed in a water tank (28 × 18 × 21 cm) filled with degassed, deionized water (NTR Systems) (Figure 1). The artery-clot preparations were positioned with the long axis of the artery parallel to the plane of the transducer. The desired ultrasound parameters were set before the experiment. The artery-clot preparations were sonicated while the mounted artery was moved through the fixed focal spot at a predetermined speed of 0.25 mm/s from one end of the artery to the other end.

Each lysis experiment was performed under real-time ultrasound imaging.

After sonication, the arteries were flushed with saline. The saline and the liquefied clots were serially filtered (400-, 50-, and 8-μm filters). The filters were then weighed to determine thrombolysis percentage.

The difference between the initial clot weight and the weight of clot particle on the 50-μm filter was defined as the lysed clot weight. The thrombolysis efficiency was calculated as the ratio between the lysed clot weight and the initial clot weight and expressed by percentage.

Optimization of Ultrasound Wave Parameter

Preliminary work with the continuous-wave mode had yielded very low thrombolysis efficiency and significant damage to the vessel wall. The use of a PW mode was studied. First, we examined the influence of 2 variables on thrombolysis efficiency, pulse length, and duty cycle. Then, we studied the combinations of 5 pulse lengths (50, 100, 200, 300, and 400 μs) and 4 duty cycles (1:10, 1:20, 1:30, and 1:40). Six experiments were performed for each set of parameters. The spatial peak time average intensity at the focal spot (Ispta) and the sonication time were kept constant (40 W/cm2 and 4 minutes, respectively).

After optimization of the pulse length and duty cycle, the relationship between Ispta and thrombolysis efficiency was evaluated (10 to 55 W/cm2 in increments of 5 W/cm2) by use of the optimized pulse length and duty cycle combination (200 μs and 1:25, respectively).

Safety Experiments

Freshly obtained swine arteries were exposed to 2 levels of HIFU, Ispta of 45 and of 90 W/cm2. At each Ispta level, sonication time was increased in 9 increments (6, 15, 25, 45, 60, 120, 300, 600, and 1800 seconds) with the optimal combinations of PW parameters (a 200-μs pulse length and a 1:25 duty cycle). Arterial segments were sonicated at 3 points (~10 mm apart) with each combination of Ispta and sonication time. Three nonsonicated arteries were used as controls.

To further explore any potential damage by HIFU, swine tissue sections (n = 18, ~3 × 3 × 3 cm), including skin and a section of the femoral artery, were prepared. These tissue sections were subjected to the safety sonication protocol previously described for the “naked” arterial segments. Nonsonicated segments were used as controls.

In Vivo Studies

The objective of the in vivo studies was to define the safety of HIFU thrombolysis in vivo. According to the guidelines set for animal research by the American Physiological Society, adult pigs (15 to 20 kg) were anesthetized with diazepam (1 mg/kg IM) and thiopentone (6 mg/kg IV). The pigs were intubated, placed on a Harvard respirator, and ventilated with halothane (1.5% to 2.5%). The ECG and blood pressure were monitored continuously throughout the procedure.

The femoral arteries were exposed to increasing levels of HIFU with optimal PW parameters (200-μs pulse length, 1:25 duty cycle). Each artery (n = 5) was sonicated at 3 points, ~10 mm apart, with the same combination of Ispta and sonication time. For the in vivo studies, a coupling Teflon cushion filled with saline...
together with gel facilitated coupling of ultrasound and adjustment of the transducer height for the specific depth of the artery from the skin to compensate for the fixed focal point. The combinations were 45 W/cm² for 25 and 45 seconds and 75 W/cm² for 25 and 45 seconds. After sonication, tissue sections (≤3×3×3 cm) including skin, vessel, and surrounding tissue were extracted and submitted to pathological examination. Non-sonicated tissue sections were used as controls.

Pathological Analysis

After sonication, the areas of sonication were carefully marked on the tissue, after which the tissues were kept in 10% neutral formaldehyde solution. Samples were dehydrated in graded alcohol, cleared in hydroxaminosole, and embedded in paraffin. Sections were cut (≤4 μm), mounted on glass slides, and stained with hematoxylin-eosin. Multiple sections from each spot of sonication were histopathologically examined by an experienced pathologist who was blinded to experimental details. The overall integrity of the vessel, continuity of the elastic structure, and damage to the intervening tissue were assessed. Damage to the vessel itself was scored according to the following criteria: grade 0, none; grade 1, intimal; grade 2, intimal and medial; and grade 3, perforation. Damage (coagulative necrosis) to the intervening tissues was scored according to the following criteria: grade 0, no coagulative necrosis; grade 1, small area of coagulative necrosis but no coagulation; grade 2, moderate area of coagulative necrosis; grade 3, large area of coagulative necrosis; grade 4, very large area of coagulative necrosis.

Statistical Analysis

Continuous variables are expressed as mean±SD. Semiquantitative variables are expressed as median (range). The relationship between PW parameters (pulse length and duty cycle) and thrombolysis efficacy was examined by 2-factorial ANOVA. The relationship between Ispta and thrombolysis efficiency was examined by the Mann-Whitney test.

Results

In Vitro

Optimization of Ultrasound Wave Parameters

Thrombolysis efficiency correlated with total sonication time regardless of the PW parameters (Figure 2). The longer duty cycle yielded better thrombolysis efficiency (Figure 3). For example, sonication with a duty cycle of 1:10 reached thrombolysis efficiency of 90±1%, compared with 45±2% when a duty cycle of 1:40 was used (P<0.0015). Furthermore, the longer the pulse length, the greater the thrombolysis efficiency, up to saturation point. The saturation point was reached earlier when a shorter duty cycle was used. For example, the use of a duty cycle of 1:10 allowed for a continuous increase of pulse length and thrombolysis efficiency up to saturation point at a pulse length of 250 μs and thrombolysis efficiency of 90±1%. With a duty cycle of 1:40, saturation was reached at a pulse length of 150 μs and thrombolysis efficiency of 45±2% (Figure 3).

In choosing the optimal pulse length and duty cycle, several aspects had to be considered: (1) a shorter duty cycle decreases the potential tissue damage, whereas a longer duty cycle achieves greater thrombolysis efficiency; (2) in shorter duty cycles, thrombolysis efficiency can be enhanced by increasing the Ispta; and (3) the use of a higher Ispta can lead to migration of the cavitation cloud outside the vessel (Figure 4). Empirical experiments yielded the correct balance between the variables: with a duty cycle of 1:25 and a pulse length of 250 μs, thrombolysis efficiency was 90%.

![Figure 3. Ultrasound thrombolysis efficiency with different duty cycles and pulse lengths. Efficacy increased as duty cycle increased. The longer the pulse length, the greater the thrombolysis efficiency, up to saturation point. The shorter the duty cycle, the earlier saturation point was achieved. All experiments were performed at Ispta of 40 W/cm².](image-url)
length of 200 μs, the cavitation cloud remained inside the vessel regardless of the levels of Ispta.

The relation between Ispta and thrombolysis efficacy was studied by use of these optimal PW parameters (Figure 5). At Ispta <25±5 W/cm², there was low thrombolysis efficacy. At Ispta >25±5 W/cm², there was a steep increase in thrombolysis efficiency. At Ispta >35±5 W/cm², an almost complete thrombolysis was achieved.

**Ultrasound Imaging Observations**

Ultrasound imaging of the thrombotic artery in the long-axis-view preparation before sonication demonstrated the vessel walls and the mild echo-dense thrombus occupying the vessel lumen (Figure 6). During sonication (Ispta >35 W/cm²), the cavitation was clearly visible as a spherical cloud of echo-dense material. When the cavitation interacted with the clot, the clot was typically shifted (≈5 mm) toward the cavitation area within ≤3 seconds. After sonication with optimal PW and Ispta parameters (duty cycle 1:25, pulse length 200 μs, and Ispta 40 W/cm²), the vessel lumen was echo-lucent and there was no sonographic evidence of residual clot or damage to the arterial wall within <2 minutes.

**Analysis of Thrombus Debris**

After use of optimal PW and intensity parameters (duty cycle 1:25, pulse length 200 μs, and Ispta 40 W/cm²), 93±1% of the lysed clots became subcapillary in size (<8 μm) (Figure 7). Higher intensity levels did not change the thrombus debris distribution profile.

**Histopathology Analysis**

In vitro, the experiments with naked arterial segments showed a structurally intact arterial wall regardless of the sonication parameters. Elastic fiber variability, which reflects the state of the vessel at harvesting, had proportions similar to those of control segments (Figure 8).

No arterial damage was observed, regardless of the parameters, in the in vitro experiments using tissue sections. Coagulative necrosis in the tissue was observed for the high ultrasound dose (Figure 9) when the sonication time was >120 seconds in Ispta of 90 W/cm² and when it was >300 seconds in Ispta of 45 W/cm².

Although the data suggested that damage score correlated with sonication time (Figure 9), only when ultrasound was...
applied for >5 minutes in 1 spot was there tissue damage, evidenced as coagulation necrosis, isolated to a well-demarcated lesion. The tissue immediately adjacent to the lesion, including the tissue between the lesion and the therapeutic transducer, was histologically normal.

In vivo, the arterial wall was structurally intact regardless of intensity and time of exposure. The structure of lipid and muscle cells was similar to that in the control segments. The morphology of the skin was unchanged from the control (Figure 10).

**Discussion**

The use of ultrasound for thrombolysis delivered either via a catheter or externally was studied in the past decade by several groups. The spectrum of acoustic energy can be separated into 2 major zones, ie, below or above the cavitation threshold. The former is the energy zone of ultrasound, used for enhancement of pharmacological thrombolysis (UPT). The latter is the zone of stand-alone ultrasound thrombolysis, as well as that for ultrasound surgery and ultrasound-assisted liposuction.

**Ultrasound-Enhanced Pharmacological Thrombolysis**

Kudo pioneered the use of external ultrasound to accelerate pharmacological thrombolysis. He showed that when used together with recombinant tissue plasminogen activator (rtPA), ultrasound can accelerate recanalization of thrombotically occluded arteries in vivo. This observation...
was later confirmed by other investigators using external ultrasound sources and catheter-based systems.11–14 It is noteworthy that whereas Kudo used ultrasound of relatively low frequencies (200 kHz), other investigators used higher frequencies (1 to 2 MHz) with subcavitation intensity. Suchkova and coworkers15 recently reemphasized the advantage of lower-frequency ultrasound (40 kHz) to optimize UPT.

It has been proposed that the mechanism of UPT is ultrasound-increased transport and uptake of rtPA into the clot.16,17 Moreover, UPT was shown not to be thermal but rather associated with transient reversible structural changes of the fibrin matrix of the clot.16–19 Importantly, UPT is not rtPA-specific and was also observed to be effective with the other thrombolytic agents urokinase and streptokinase.20,21

Ultrasound Thrombolysis

UPT requires the presence of a thrombolytic agent in a therapeutic dose. Thrombolytic agents have significant limitations, including complications and high cost.22 Because of these limitations, attempts have been made to use ultrasound for thrombolysis as stand-alone therapy, ie, without adjunct use of pharmacological thrombolytic agents.

Experimentally, high-power, low-frequency ultrasound was found to selectively lyse thrombi with a wide margin of safety. The ultrasound intensity required to lyse thrombi is \( \frac{1}{20} \) of that required to induce arterial wall damage.23 The resistance of arteries to ultrasound-induced damage was also noted in ultrasound surgery and ultrasound-assisted lipoplasty experiences.24,25 Finally, the resistance of arteries to ultrasound-induced damage was noted to diminish with increased ultrasound intensity.26

Figure 8. A, Representative histological section of arterial segment exposed in vitro to high-power focused ultrasound. Focal area was positioned within vessel lumen. B, Details of arterial wall, which is structurally intact. Elastic fiber variability, which reflects state of vessel at harvesting, is similar to control segment.

Figure 9. Damage as function of sonication time. Damage increases in same spot above threshold of sonication of 60 seconds in low \( I_{\text{total}} \) (45 W/cm²) and of 300 seconds in high \( I_{\text{total}} \) (90 W/cm²).

Figure 10. Representative reconstructed histological sections of artery and its surrounding tissues exposed in vivo to high-power ultrasound. Transducer was located in contact with skin. Focal area was within arterial lumen (dotted circle) A, Arterial wall, lipid cells, and striated muscle are intact. B, Skin morphology did not change vs control.
We have studied catheter-based ultrasound thrombolysis. Our experience in acute myocardial infarction, acute coronary syndromes, and occluded saphenous vein grafts suggests that high-power, low-frequency ultrasound delivered in a catheter-based system can achieve effective and safe thrombolysis.\(^1\)\(^2\) Similarly encouraging results were reported by Hamm et al,\(^27\) who used a different catheter-based ultrasound delivery system to lyse clots in patients with acute myocardial infarction. The risk of distal embolization after ultrasound thrombolysis was extensively addressed. In vitro, Hartnell et al \(^28\) demonstrated that most of the debris, consisting of platelet and red blood cell aggregates, are of subcapillary size, an improvement that was not observed in thrombolytic- and PTCA-treated patients.\(^29\)\(^30\)

We hypothesized that an external high-power, low-frequency ultrasound source can focus the energy from a distance to a clot within the body and achieve thrombolysis with no damage to structures lying in the path of the ultrasound beam. In this study, we investigated in vitro external HIFU thrombolysis using 500-kHz pulsed ultrasound guided by ultrasound imaging. The use of the correct PW parameters was critical to the success of the system operation: external therapeutic ultrasound was found to be very efficient in inducing rapid thrombolysis with no damage to the arteries or intervening tissue. There was no need for adjunct pharmacological agents. The generated debris was mostly (93% of clot mass) subcapillary in size. It has been proposed that the mechanism of ultrasound thrombolysis is by nonselective disruption of the thrombus fibrin matrix by the imploping cavitations.\(^8\)\(^23\)\(^31\) We identified tissue damage when higher intensity levels (\(\geq45\) W/cm\(^2\)) were applied only for a relatively long time (>5 minutes), guaranteeing a potentially wide therapeutic index. Furthermore, the tissue damage was limited to the focal point, with none occurring to the intervening tissue.

When the optimal operating parameters are used, external ultrasound can generate and sustain a stable field of cavitation. The ability to better control the cavitation effect by a monochromatic ultrasound technique yields a very effective thrombolysis compared with the effect of shock waves, in which the transient cavitation effect bore only modest thrombolytic efficiency.\(^8\)

Other investigators have attempted external ultrasound thrombolysis. Because they used nonfocused, nonpulsing systems and no visual guidance, they were limited in their ability to accurately deliver therapeutic levels of ultrasound. The limitation was circumnavigated elegantly by the use of echo-contrast material, which lowers the cavitation threshold.\(^32\) Nevertheless, thermal injury was a significant safety problem that had to be addressed by a tissue cooling system.\(^33\)\(^34\)

**Conclusions**

The results of this study suggest that external HIFU thrombolysis may be a safe and effective method to induce thrombolysis. In addition, ultrasound therapy can be guided by ultrasound imaging to identify and optimize the acoustic window, aim the therapeutic ultrasound, ensure generation of cavitation, and monitor therapy. More clinical work is needed to further evaluate this method and define its clinical feasibility and potential risks to intervening tissues whose acoustic properties are different from those of soft tissue, e.g., lung tissue in coronary applications and skull for intracranial applications.

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**References**


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