Effect of externally applied focused acoustic energy on clot disruption in vitro

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ABSTRACT

Application of low-frequency ultrasound for clot disruption has been suggested as a potential therapy to enhance thrombus dissolution, but the optimal mode for delivery of ultrasound with clot-disruptive properties has not yet been extensively explored. Target-specific effects are desirable and may be accomplished by focusing the ultrasound. Adequate focusing, however, requires a short wavelength. The aim of this study was to compare the clot-disruptive effects of different modalities of focused acoustic energy. An in vitro model (10 blood clots for each modality) was used to test the clot-disruptive capacity of (i) shock waves generated in an electrohydraulic lithotriptor; (ii) focused continuous ultrasound of frequency 1.1 MHz, delivered from a specially constructed piezoelectric transducer; and (iii) focused pulse-modulated ultrasound of frequency 1.1 MHz delivered from the same transducer. Exposure to 30 s of focused pulse-modulated ultrasound caused a marked reduction (99 ± 2%) in clot weight compared with 30 shock waves (11 ± 5%) or 30 s exposure to focused continuous wave ultrasound (11 ± 6%)(P < 0.0001). The observed marked and rapid disruptive effect on blood clots of focused high-frequency ultrasound indicates an alternative approach for external ultrasound-mediated thrombus destruction in vivo. The focused pulse-modulated technique has potential to exhibit the desired effect in a well-defined target volume and provides the means for control of the average power.

INTRODUCTION

Thrombolytic therapy is commonly used to recanalize acutely occluded coronary arteries. It reduces mortality [1–5] and is known to preserve left ventricular function [3,6,7]. Nevertheless, the need for adjunctive therapy has been pointed out in recent reviews [8,9]. Application of ultrasound energy for clot disruption is such a potential therapy to enhance thrombus dissolution. Catheter-delivered low-frequency (15–30 kHz, wavelength in tissue approximately 10–5 cm) ultrasonic energy has been shown to dissolve clots in vitro [10–16], in animal models [12,15,17] and in patients [17–19] even without the combination with thrombolytic agents. Enhancement of the effect of thrombolytic agents by exposure of external ultrasound energy has been reported in vitro [20–27] and in animal models [21,28,29], whereas the information on clot-disrupting effects of external ultrasound per se is quite sparse [30]. All these observations indicate the possibility of non-pharmacological clot disruption in patients with peripheral as well as coronary arterial thrombotic occlusions by transcutaneous exposure of ultrasound energy.

Before clinical application of this technique, however, a number of limitations have to be overcome. Although low-frequency ultrasound has been shown to be more

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efficient than higher frequencies [31], the optimal mode for delivery of ultrasound with clot-disruptive properties has not yet been extensively explored. High-intensity, low-frequency ultrasound was reported to be associated with a substantial skin injury in a rabbit model [29], and since there is a substantial path distance between the thrombus and the ultrasound probe in the clinical situation, the remaining ultrasound intensity at site may not be enough to disrupt the thrombus in that situation. Another limitation with the low-frequency approach is the difficulty achieving adequate focusing of the sound due to the long wavelength. On the other hand, an inherited difficulty with high-intensity focused ultrasound is the phenomenon of ‘acoustic shielding’, i.e. the progressive build up of microbubbles along the path of the ultrasound wave hindering (by extinction) the sound from reaching its desired target volume [31]. This phenomenon may be diminished by pulsing the ultrasound [32]. Two important features of externally delivered ultrasound for thrombus disruption are (i) ability to deliver the effect into a specified limited target volume at a considerable distance from the transducer, and (ii) capacity to carry out its effect at a low average power.

Based on the hypothesis that the mode of ultrasound delivery to the target volume may be an important determinant of its efficacy, we used an in vitro model to test the clot-disruptive capacity of (i) shock waves generated in an electrohydraulic lithotriptor; (ii) focused continuous ultrasound of frequency 1.1 MHz, delivered from a specially constructed piezoelectric transducer; and (iii) focused pulse-modulated ultrasound of frequency 1.1 MHz delivered from the same transducer.

### METHODS

#### Clot preparation

Whole blood was obtained by antecubital venepuncture from healthy volunteers in the morning after fasting for 12 h. The blood was allowed to coagulate in a closed horizontal glass test tube at room temperature for 1 h. The resulting clot (100 mm long) with an almost perfect semicircular profile (13 by 6.5 mm) was carefully removed from the tube in one single piece and subsequently cut into slices approximately 5-mm thick yielding clot weights in the range of 260–400 mg (see Figure 1). Each clot was weighed on a precision scale and submerged with 0.5 ml of normal saline into a thin vinyl condom to a minimum. The clot was then manually positioned in the clearly visible focus of the ultrasound cone converging exactly at the surface of the water.

#### Ultrasound system

We used an experimental piezoelectric focused ultrasound transducer constructed by us previously [31]. The transducer consisted of 16 slightly damped piezoelectric curved ceramic plates (size 60 × 60 × 2 mm, focal distance 170 mm; PZ26, Ferroperm, Denmark). The half-wavelength resonance frequency is 1.1 MHz. The damping was adjusted to match the modulation side bands. The transducer aperture diameter is 24.5 cm and the curved radiating area is approximately 560 cm². Since the focal distance is 17 cm, the sound cone-angle will therefore be close to 90°. The −6 dB (100–50% pressure as dB) focal volume at 1.1 MHz is approximately 0.01 cm³. Some silicon bellows were mounted around the bowl; these can be used to vary the depth of the focus in tissue. As coupling media, degassed tap water was used, rest concentration O₂ < 3 mg/L. The amplifier bandwidth (0.3–35 MHz) permits actual side bands.

The transducer was fed by a signal source via a linear amplifier. The bowl is filled with degassed water so that the focus hits the surface of the water. The clot, placed in a thin condom filled with a small amount of normal saline and closed by haemostatic forceps, was manually placed in the acoustic focus for 30 s. The clot shape and size are illustrated in the upper left corner.

#### Lithotriptor

The electrohydraulic lithotriptor Lithocut C 3000 ESWL (Comair, Stockholm, Sweden) was used for the experiments. Lithocut has an aperture diameter of 24 cm, a maximal treatment depth of 13 cm and a cone angle of 90°. The maximal electric energy per shot is 22.5 J.
developed radiation force. The average power is thus approximately 30 W.

**Clot disruption protocol**

Ten clots were sonicated with each acoustic modality in a randomized order. The condom was adopted into the clearly visible focal volume of either system during the exposure (Figure 1). Each clot was exposed for 30 s in the experiments using continuous or pulse-modulated focused ultrasound. In the lithotriptor each clot was exposed to 30 shock waves during a total time of 60 s [32]. The remaining clot was removed from the tube in one piece and weighed on the same precision scale. The high-viscosity fluid in the tube was poured on to filter paper to detect any remaining fragments.

**Statistics**

Results are given as means and standard deviation. Differences between groups at baseline were calculated with one-factor ANOVA. Treatment effects were analysed by repeated measures ANOVA with ultrasound modalities as between factor, and before and after treatment as within factor.

**RESULTS**

Table 1 summarizes the reduction in thrombus weight, and Figure 2 shows the percentage thrombus disruption with each ultrasound mode. Exposure to 30 s of focused pulse-modulated ultrasound caused a marked reduction (99 ± 2%) in clot weight compared with 30 shock waves or 30 s exposure to focused continuous wave ultrasound (11 ± 5% and 11 ± 6% respectively). There was no difference in initial clot weight between groups. The weight reduction with ultrasound sonication was significant in all groups (P < 0.0001).

The effect of the different modalities of ultrasound was consistent within each modality, showing similar effects on all 10 clots in the respective group. With pulse-modulated ultrasound 9 of 10 clots were completely disrupted with one 20 mg fragment remaining from one clot. The other tubes in this group contained only a highly viscous fluid with no detectable fragments. Shockwave treatment and exposure of continuous focused ultrasound resulted in one remaining clot in each tube without any sign of fragmentation. The weight range of the remaining clot is given in Table 1. During the course of the experiment the clot age increased by approximately 2 h. There was no apparent relationship between the degree of clot disruption and clot age.

**DISCUSSION**

This is the first study to report the effect of different ultrasound modalities including focused ultrasound on thrombus dissolution. By modulating the duration of the pulse and pause of the high-frequency ultrasound we were able to achieve marked effects on the blood clot within a very limited time frame and, equally important, with a low average power.

The most likely and dominant mechanism for thrombus disruption is believed to be acoustic cavitation. Acoustic cavitation results in the formation of microscopic bubbles when ultrasound waves pass through a liquid with an alternating pressure. The cavitation may be either stable or transient. Stable bubbles may oscillate for many cycles of the sound pressure before collapsing whereas transient cavities generally exist for less than one cycle. Both the oscillation of microbubbles and their

**Table 1  Thrombus reduction after exposure to different modes of focused ultrasound**

<table>
<thead>
<tr>
<th>Ultrasound mode</th>
<th>Thrombus weight (mg)</th>
<th>Pre-ultrasound</th>
<th>Post-ultrasound</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock wave from lithotriptor</td>
<td></td>
<td>345 ± 30 (310–400)</td>
<td>306 ± 25 (280–380)</td>
<td>39 ± 23 (10–90)</td>
</tr>
<tr>
<td>Focused continuous at 1.1 MHz</td>
<td></td>
<td>337 ± 41 (260–390)</td>
<td>300 ± 39 (230–360)</td>
<td>37 ± 16 (10–60)</td>
</tr>
<tr>
<td>Focused pulse-modulated at 1.1 MHz</td>
<td></td>
<td>334 ± 37 (280–400)</td>
<td>2 ± 6 (0–20)</td>
<td>332 ± 39 (280–400)</td>
</tr>
</tbody>
</table>
rapid collapse in the acoustic field result in high local pressure changes. This mechanical shock may be felt a few radial distances from the collapsed cavitation [33]. An intense shear stress may occur in the tissue in the immediate vicinity of the collapsing bubble which could alter the erythrocyte membrane, break fibrin bonds, affect platelets and finally lead to thrombus disruption.

The observed superiority of pulse-modulated focused ultrasound compared with continuous ultrasound indicates that the degree of acoustic shielding was minimized by the relatively long pauses between the short pulses allowing a sufficient cavitation to occur in the focus. The high mechanical resistance of the blood clots to shock waves which readily fragment kidney stones or ceramic model stones is probably due to their respective structures.

Using an ultrasound system with a fixed power intensity (2.9 W/cm²), Luo et al. [30] reported greater thrombus dissolution with lower frequencies than with higher frequencies of ultrasound. At a frequency of 25 kHz, thrombi with an initial weight of approximately 260 mg were almost completely (99%) dissolved after 3 min of exposure. Olsson et al. [27] showed that 170 kHz is more effective than 1 MHz for thrombus dissolution. These data are supported by known observations of microbubble behaviour. The size of bubbles generated by ultrasound during cavitation is inversely proportional to the ultrasound frequency. The larger bubbles produced by the lower frequencies of ultrasound are thought to produce greater force during their oscillation and implosion than the smaller bubbles produced at higher frequencies. As ultrasound frequency is increased, more power is necessary to produce cavitation [34]. In another study using a similar clot weight, 30 min of pulsed non-focused ultrasound (0.5 MHz) in combination with streptokinase yielded a clot weight reduction of 15.1% [35]. However, with the present pulsed focused technique we show apparently much more rapid clot destruction in spite of the higher carrier frequency and slightly higher initial clot weight. This apparent difference in the results is compatible with a significant impact of the previously described shielding phenomenon on the efficacy of acoustic clot disruption through water and most probably tissue.

CONCLUSION

The cited properties of ultrasound have led most researchers to explore the lower frequencies in the quest for a clinically applicable ultrasound-generated thrombus disruption. We believe that a major prerequisite for treatment of intravascular clots in the clinical setting is the ability to guide the ultrasound energy to the immediate vicinity of the thrombus. We have therefore restricted our attention to higher frequencies which can be focused into a small volume. Although there are similarities with the extracorporeal shock wave lithotripsy approach for bringing the acoustic energy into the patient’s body (shock wave pulses have been shown to propagate well into the patient’s soft tissue without causing thermal skin injury), it is not clear whether the modality modifications necessary for rapid clot destruction that we have described will allow thrombus destruction in deep tissues (50–100 mm) without causing unacceptable damage to the surrounding tissues. A more readily accomplished application for this technique is probably as an adjunct therapy to peripheral clinical thrombolysis. Nonetheless, the marked and rapid disruptive effect observed on blood clots in our study with a technique potentially capable of concentrating its intensity to a very limited target volume deep inside the body at a low-level average power indicates an alternative approach for external ultrasound-mediated thrombus destruction in vivo, which warrants further exploration.

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Thrombus disruption by acoustic focused energy


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