Brief Rapid Communications

Noninvasive In Vivo Clot Dissolution Without a Thrombolytic Drug
Recanalization of Thrombosed Iliofemoral Arteries by Transcutaneous Ultrasound Combined With Intravenous Infusion of Microbubbles

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Background—Previous in vivo studies have shown that microbubbles not only enhance the effectiveness of thrombolytic agents in the presence of ultrasound but may also augment clot dissolution without thrombolytic drugs.

Methods and Results—The objective of this study was to examine the efficacy of arterial clot disruption by a noninvasive, nonlytic approach with intravenous administration of perfluorocarbon-exposed sonicated dextrose albumin (PESDA) and transcutaneous delivery of ultrasound alone. Pairs of iliofemoral arteries in 10 rabbits were randomized to receive transcutaneous ultrasound treatment or no ultrasound treatment after an acute artery thrombotic occlusion and intravenous PESDA infusion. Five arteries from 3 additional rabbits served as controls (ultrasound alone). All 10 iliofemoral arteries treated with PESDA+ultrasound were recanalized by angiography after ultrasound treatment. None of the 10 contralateral arteries treated with PESDA alone and none of the 5 arteries treated with ultrasound alone were patent after 1 hour. D-Dimer levels did not change after intravenous PESDA+ultrasound–mediated reperfusion.

Conclusions—In vivo arterial clot dissolution can be achieved with intravenous microbubbles and transcutaneous ultrasound delivery alone. This technique has potential for clinical application in patients with acute arterial and venous thrombotic occlusions. (Circulation. 1998;97:130-134.)

Key Words: thrombosis ■ ultrasonics ■ occlusion

Although thrombolytic therapy is effective, there is a need for simpler, safer, more inexpensive, and noninvasive methods for rapid and effective recanalization of thrombosed arteries that can be initiated immediately upon arrival to the hospital or even en route.

Recently, it has been shown that perfluorocarbon-exposed sonicated dextrose albumin (PESDA) microbubbles alone enhance clot disruption with ultrasound in an in vitro model. In the present study, we evaluated a noninvasive approach to recanalize in vivo thrombosed arteries by using a combination of transcutaneous ultrasound with intravenous administration of PESDA microbubbles, avoiding the administration of a fibrinolytic agent.

Methods

Animal Preparation

Thirteen adult New Zealand White rabbits weighing 3.2 to 5.3 kg were anesthetized and maintained with ketamine (20 mg/kg) and xylazine (3.0 mg/kg) intravenously and on one side the artery was randomized to ultrasound alone or no ultrasound for 15 minutes. After angiographic documentation of bilateral thrombotic occlusions, PESDA was administered intravenously, and the rabbit was followed with repeated bilateral angiography every 15 minutes up to 60 minutes to assess patency of each artery. In the transcutaneous ultrasound control group (no PESDA), after 60 minutes of ultrasound delivery to the first artery, the contralateral artery was exposed to ultrasound in the same fashion. Each angiogram was analyzed by the consensus of four reviewers for the presence or absence of occlusion, vessel spasm, and distal or side branch embolization.

The method used to induce arterial thrombus has been previously described. Occlusion was induced in both iliofemoral arteries in a random order.

Experimental Protocol

After angiographic documentation of bilateral thrombotic occlusions of the iliofemoral arteries in 10 rabbits, PESDA was administered intravenously and on one side the artery was randomized to ultrasound therapy. The contralateral artery served as a control (exposed to PESDA without ultrasound). Transcutaneous ultrasound alone (a control without administration of PESDA) was applied to 3 rabbits (PESDA without ultrasound). Transcutaneous ultrasound alone (a control without administration of PESDA) was applied to 3 rabbits (PESDA alone). After ultrasound treatment, the PESDA microbubbles were recanalized by angiography after ultrasound treatment. None of the 10 contralateral arteries treated with PESDA alone and none of the 5 arteries treated with ultrasound alone were patent after 1 hour. D-Dimer levels did not change after intravenous PESDA+ultrasound–mediated reperfusion.

Conclusions—In vivo arterial clot dissolution can be achieved with intravenous microbubbles and transcutaneous ultrasound delivery alone. This technique has potential for clinical application in patients with acute arterial and venous thrombotic occlusions. (Circulation. 1998;97:130-134.)

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Intravascular ultrasound imaging (3.5F catheter, 30-MHz transducer; Boston Scientific Corp and Hewlett-Packard Sonos Intravascular Imaging System) was performed in one rabbit treated with PESDA to assess (1) if intravenously administered PESDA microbubbles cross the lungs and reach the arterial circulation and (2) how long after injection the microbubbles can be visualized in the arterial circulation (aorta). Images were recorded at baseline, before administration of PESDA, every 1 minute for 10 seconds during the first 15-minute ultrasound delivery, and at the beginning of the second 15-minute ultrasound delivery.

Plasma D-dimer levels (Diagnostica Stago) were measured in 5 rabbits. Samples were obtained after thrombus induction, after ultrasound and PESDA therapy resulted in angiographic arterial patency, and 1 hour after 1000 units of heparin was administered intravenously.

Perfluorocarbon-Exposed Sonicated Dextrose Albumin
The preparation of PESDA microbubbles was described previously.1-3 One milliliter of PESDA was infused slowly over 5 minutes through the ear vein catheter at the beginning of the first 15 minutes of ultrasound delivery.

Ultrasound Device
An ultrasonic generator (Cybersonics) was used that operates at ≈37 kHz and uses both pulse and sweep frequencies, 91 Hz and ±1 kHz, respectively. The power can range to 160 W and the peak negative pressure is 103 kPa (1.03 bAr). The ultrasound transducer was applied transcutaneously over the arterial occlusion site, which was marked on

Figure 1. Case example of repeated angiography of the both iliofemoral arteries. a and b, Bilateral thrombotic occlusions of the arteries; c, artery treated with intravenous perfluorocarbon-exposed sonicated dextrose albumin (PESDA) and transcutaneous ultrasound (recanalized after 30 minutes of therapy), which remained patent 60 minutes after intravenous heparin; and d, control side, treated with PESDA alone, remained occluded.
the skin with a metallic marker that was positioned at the time of angiography.

Pathological Studies
At the end of each experiment, rabbits were euthanized by an intravenous injection of KCl. The iliofemoral arteries, ultrasound-exposed skin, and soft tissues in all rabbits were excised, examined grossly, and then fixed in 10% neutral buffered formalin for 24 to 72 hours. The iliofemoral arteries were then cut transversely every 2 mm for the length of the vessel and processed as previously described.4,5 The arteries were examined in random order, with the examiner blinded to whether the artery was treated with ultrasound.

Statistical Analysis
Data are given as mean value±SD. The χ² test was used to compare the angiographic patency rate among the different treatment groups. A value of P<.05 was considered statistically significant.

Results
Thirteen rabbits were included in the study, 10 in the intravenous PESDA+unilateral ultrasound exposure group and 3 in the ultrasound-alone control group.

Angiographic Results
All 10 arteries treated with ultrasound and PESDA reperfused by angiography within 60 minutes of ultrasound delivery, 3 of them (30%) were recanalized after one 15-minute treatment period, 2 more arteries (20%) reperfused after the second 15-minute of (30 minutes) ultrasound therapy, and an additional 2 after the third 15-minute (45 minutes) ultrasound therapy period. The remaining 3 arteries recanalized after a cumulative time period of 60 minutes. In contrast, none of the contralateral arteries exposed to PESDA without ultrasound reperfused during the same period (P=.00006). None of the 5 control arteries that were treated with 60 minutes of transcutaneous ultrasound without PESDA reperfused (P<.000004 for the difference among groups). Fig 1 shows an example of an angiogram of rabbit iliofemoral artery treated with PESDA and ultrasound after induction of a thrombotic occlusion (a) and after recanalization (c).

After the initial recanalization of the ultrasound-treated arteries, heparin was administered intravenously and arterial patency was assessed by repeated angiography every 15 minutes for a total of 1 hour. All of the 10 arteries that recanalized after ultrasound therapy remained patent during this period. However, 3 of the arteries treated with PESDA without ultrasound recanalized after administration of heparin: 1 artery 15 minutes after heparin administration and 2 arteries 30 minutes after heparin was infused.

In one rabbit in the PESDA group, distal embolization with an occlusion of a side branch was evident after ultrasound reperfusion. In this rabbit, the contralateral artery recanalized after heparin administration; angiography also revealed distal embolization in this artery.

Intravascular Ultrasound Imaging
After intravenous injection of PESDA microbubbles, there was opacification of the aorta for >10 minutes, confirming that the PESDA crossed the lungs, recirculated in the blood, and was present at the site of thrombotic occlusion.

Plasma D-Dimer Levels
After induction of bilateral thrombotic iliofemoral arterial occlusions, the mean D-dimer level was 5500±3535 ng/mL; after combined transcutaneous ultrasound and PESDA induction of unilateral arterial patency, the mean D-dimer level was 2650±3008 ng/mL, and 1 hour after a bolus of 1000 units of heparin and monitoring to confirm arterial patency, the mean D-dimer level fell to 800±410 ng/mL.

Histopathology
Fig 2A demonstrates a patent iliofemoral artery after exposure to ultrasound+PESDA. Histological evaluation revealed that 9 of 10 arteries treated with ultrasound+PESDA were patent; these 9 arteries had minimal residual thrombus (≤25% cross-sectional area obstruction by thrombus). However, 1 ultrasound+PESDA-treated artery was occluded by thrombus. As shown in the Table, this artery only had 15 minutes of exposure to ultrasound, whereas all the other arteries exposed to ultrasound+PESDA had ≤25% of the arterial cross-sectional area occupied by residual thrombus. Three arteries exposed to PESDA alone that recanalized by angiography after heparin administration were patent by microscopy. The other 7 arteries exposed to PESDA alone remained occluded. Fig 2B
demonstrates a thrombosed iliofemoral artery that had been exposed to PESDA alone, and it failed to recanalize. Microscopic areas of necrosis were found in the arterial wall in all vessels. The magnitude of vessel injury was the same in the ultrasound-treated arteries as in the control arteries. There was no evidence of damage or inflammation in the skin or soft tissues overlying the ultrasound-treated iliofemoral arteries.

**Discussion**

In this rabbit study of a thrombotically occluded iliofemoral artery, all arteries treated with the combination of PESDA and ultrasound recanalized angiographically within 60 minutes of therapy. Ultrasound and PESDA were both necessary, since none of the arteries exposed to ultrasound alone or PESDA alone recanalized within this period. Furthermore, ultrasound-induced thrombus disruption was not associated with reocclusion (as documented by repeated angiography) after recanalization and did not result in an elevation of D-dimer levels, and therefore was not associated with activation of the fibrinolytic system.

Since Tachibana and Tachibana demonstrated in vitro that the room air-filled albumin microbubbles (Albunex, Molecular Biosystems, Inc.) accelerate the clot-dissolving effects of ultrasound energy, the clinical potential for microbubbles to produce clot dissolution has been further elucidated. Nishioka et al., using a similar rabbit iliofemoral artery model and a 20-kHz ultrasound transducer, were able to reperfuse (TIMI flow grade II or III) 13 out of 17 (76%) of the arteries with a combination of transcutaneous ultrasound and direct injection of dodecafluoropentane (Sonus Pharmaceuticals, Inc) emulsion into the iliofemoral artery. However, thermal damage induced by the ultrasound exposure to the overlying skin and subcutaneous soft tissues was a major problem. Moreover, in that experiment, the microbubbles were delivered by a more invasive intra-arterial injection. In the present study the microbubbles were administered into a peripheral vein without the loss of their effectiveness in enhancing clot disruption.

The mechanism of clot dissolution with ultrasound and PESDA is still unclear. One possibility is that the microbubbles provide a nucleus for cavitation. This would lead to microstreaming, which could produce a “shearing away” of the thrombus. This possibility is supported by the observation that D-dimer levels did not change after ultrasound and PESDA, indicating that fibrinolysis was not induced. This could also explain why microbubbles enhance the effect of a thrombolytic agent in the presence of ultrasound. By shearing away the clot, more fibrin is exposed to the lytic agent. A direct effect of the dextrose and albumin solution is unlikely because none of the arteries treated with PESDA alone recanalized.

This new method of treating arterial thrombosis also appears to be safe. The ultrasound device used in this study emits a concentrated ultrasound beam, in a pulsed mode, at 37 kHz, and has a cooling manifold over the skin surface. There was no histological evidence of ultrasound-mediated damage to overlying skin or soft tissues, as was observed in previous studies. In this model, we did not examine the long-term effects of transcutaneous ultrasound on the tissues. Although pathological changes were not detected by light microscopy, ultrastructural changes cannot be ruled out. Distal embolization was

**Pathological Findings**

<table>
<thead>
<tr>
<th>No.</th>
<th>Mode</th>
<th>Reperfusion</th>
<th>Residual Thrombus, %</th>
<th>Necrosis % of Arterial Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>USD + PESDA</td>
<td>45 min</td>
<td>≤25%</td>
<td>≤25%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>≤25%</td>
</tr>
<tr>
<td>2.</td>
<td>USD + PESDA</td>
<td>15 min</td>
<td>≤25%</td>
<td>≤25%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>≤25%</td>
</tr>
<tr>
<td>3.</td>
<td>USD + PESDA</td>
<td>60 min</td>
<td>≤25%</td>
<td>≤25%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>≤25%</td>
</tr>
<tr>
<td>4.</td>
<td>USD + PESDA</td>
<td>15 min</td>
<td>100%</td>
<td>≤25%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>26-50%</td>
</tr>
<tr>
<td>5.</td>
<td>USD + PESDA</td>
<td>60 min</td>
<td>≤25%</td>
<td>26-50%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>≥76%</td>
</tr>
<tr>
<td>6.</td>
<td>USD + PESDA</td>
<td>30 min</td>
<td>≤25%</td>
<td>26-50%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>after heparin</td>
<td>≤25%</td>
<td>≤25%</td>
</tr>
<tr>
<td>7.</td>
<td>USD + PESDA</td>
<td>60 min</td>
<td>≤25%</td>
<td>26-50%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>26-50%</td>
</tr>
<tr>
<td>8.</td>
<td>USD + PESDA</td>
<td>45 min</td>
<td>≤25%</td>
<td>51-75%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>after heparin</td>
<td>≤25%</td>
<td>51-75%</td>
</tr>
<tr>
<td>9.</td>
<td>USD + PESDA</td>
<td>15 min</td>
<td>≤25%</td>
<td>≤25%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>after heparin</td>
<td>51-75%</td>
<td>≤25%</td>
</tr>
<tr>
<td>10.</td>
<td>USD + PESDA</td>
<td>30 min</td>
<td>≤25%</td>
<td>≤25%</td>
</tr>
<tr>
<td></td>
<td>PESDA alone</td>
<td>none</td>
<td>100%</td>
<td>≤25%</td>
</tr>
</tbody>
</table>

USD indicates ultrasound; PESDA, perfluorocarbon-exposed sonicated dextrose albumin.
evident in only one ultrasound-treated artery. In this rabbit, distal embolization was also present in the contralateral artery that reperfused after heparin administration.

Conclusions
This is the first study to demonstrate in vivo a noninvasive, nonlytic approach for clot dissolution with the use of intravenous microbubbles and transcutaneous ultrasound. Our findings show that transcutaneous ultrasound combined with PESDA is an efficient, rapid, and safe method for clot dissolution. We conclude that transcutaneous ultrasound in combination with PESDA could be an alternative to systemic lytic therapy in patients with acute arterial and venous thrombotic occlusions.

Acknowledgments
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References
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