Does External Ultrasound Accelerate Thrombolysis?

Results From a Rabbit Model

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**Background** Prior in vitro and in vivo studies have reported that external ultrasound accelerates thrombolysis at intensities too low to have a direct effect on clot dissolution in the absence of a thrombolytic agent. The present study was undertaken to examine the ultrasound effect on thrombolysis and reocclusion in a rabbit thrombosis model.

**Methods and Results** Blood clots were produced in a femoral artery segment with endothelial damage and distal stenosis. Recombinant tissue-type plasminogen activator (rTPA) was infused at 30 μg·kg⁻¹.min⁻¹ for 60 minutes. Femoral artery flow was measured every 5 minutes for 2 hours. Rabbits were randomized to four groups with continuous wave ultrasound on or off with or without intravenous injection of 17 mg/kg aspirin (+US/−US/+Asp/−Asp). Ultrasound frequency and intensity were 1 MHz and 6.3 W/cm². In seven of eight and five of five rabbits given rTPA and −US/−Asp or −US/+Asp, respectively, reflow was observed, persisting to the end of the observation period. In five of nine and four of five rabbits given rTPA and +US/−Asp or +US/+Asp, reflow was achieved, but persistent reocclusion was subsequently observed in five of five and two of four of these rabbits, respectively. Overall, femoral artery patency was worse and reocclusion occurred more often when ultrasound was added to rTPA (P=.002 by nonparametric ANOVA). However, initial reflow occurred more rapidly with ultrasound exposure (21±10 and 33±6 minutes for the +US/+Asp and +US/−Asp groups, respectively) compared with without ultrasound (46±13 and 74±14 minutes for the −US/+Asp and −US/−Asp groups, respectively) (P=.03 by ANOVA).

**Conclusions** Although time to initial reflow was shortened by ultrasound, it was associated with less reperfusion and more reocclusion in this model. A possible explanation for these results is ultrasound-induced platelet activation counterbalancing its thrombolysis-accelerating effect. (*Circulation*. 1994;89:339-344.)

**Key Words** rTPA • aspirin • thrombosis • reocclusion

Thrombotic occlusion of the arterial lumen is the main pathophysiological event that causes myocardial infarction as well as peripheral artery occlusion.¹⁻³ Rapid reestablishment of flow is the best way to maintain viability of the supplied territory.⁴⁻⁵ Thrombolytic therapy is designed to achieve this goal, and it has been found to reduce mortality as well as preserve myocardial function in myocardial infarction survivors.⁶⁻⁷ However, to achieve myocardial salvage, coronary blood flow must be restored rapidly.⁶ Thrombolytic results are often limited by the duration needed to achieve recanalization as well as by thrombotic reocclusion of the recanalized artery.⁸ Pharmacological and mechanical adjuncts can be combined with thrombolytic therapy to optimize its results and achieve a rapid and sustained recanalization.⁹

One of the means by which thrombolysis might be accelerated is by exposure to external ultrasound energy.¹⁰⁻¹⁷ Prior in vitro and in vivo studies have reported that external ultrasound accelerates thrombolysis at ultrasound intensities that are too low to have a direct effect on clot dissolution in the absence of a thrombolytic agent.¹⁰⁻¹⁷ However, earlier studies also revealed a potential for platelet activation and thrombosis induction by ultrasound exposure in vitro.¹⁸⁻²¹ This latter property might be hazardous during thrombolysis.

The purpose of this study was, therefore, to examine the ultrasound effect on thrombolytic recanalization and reocclusion in a rabbit femoral artery thrombosis model.

**Methods**

**Animal Model**

Twenty-seven New Zealand White rabbits weighing 3.5 to 5.0 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg followed by 10 mg at 30- to 60-minute intervals) via the marginal ear vein (Fig 1). The auricular artery was cannulated for blood sampling. The right femoral artery and vein were exposed, and side branches were ligated except for the right superficial epigastric artery, which was cannulated with a 24-gauge cannula for local thrombus induction. An electromagnetic flowmeter probe (Nihon Inc) was positioned proximally, and arterial blood flow was measured throughout the experiment. A stepwise incision was produced distally by constricting the artery with a 4.0-silk suture to reduce the flow by 40% (stenotic flow). Then, a 1-cm segment of the femoral artery was clamped distally and proximally to the superficial epigastric artery insertion, and the isolated segment was emptied by the cannulated side branch. The isolated segment was traumatized by three repeated external compressions with blunt forceps to produce endothelial injury. Ten units of bovine thrombin (Thrombimax, Armour Pharmaceutical Co, Kaukaakee, Ill) was mixed with 0.1 mL of fresh

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blood drawn from the auricular artery and injected into the isolated femoral artery segment through the right epigastric artery cannula. Ten minutes later, the proximal and then the distal clamps were released for 15 seconds and then reclamped for an additional 5 minutes. The second clamping was used to ensure complete occlusion of the artery because residual flow frequently is observed after the initial clamp release. The absence of blood flow was monitored with the flow probe for 10 minutes after the second clamp release to confirm a stable thrombotic occlusion of the femoral artery (flow=0).

Ultrasound Apparatus and Calibration

The ultrasound source was a Sonopulse 407 instrument (Enraf Nonius Delft, Delft, The Netherlands) used for physical therapy. The ultrasound frequency was 1 MHz. The intensity of the acoustic field from this transducer was calibrated by a hydrophone in a water tank.

The cylindrical transducer, which was 3 cm in diameter, was positioned 10 to 15 cm above the arterial clot. We wanted to insonify the clot at an axial distance from the transducer representing the transition between the near field and far field—approximately 14 to 15 cm. To achieve this, a “standoff” was created to ensure fluid continuity between the ultrasound transducer and the clot by using a latex condom filled with saline with its tip submerged in a saline-filled pouch at the femoral clot site (Fig 1). The pouch was created at the right femoral site by dissecting and tenting up the rabbit skin with four 2-0 cutaneous silk sutures that were tied to a ringstand above the surgical site. With this technique, a pouch of about 5 cm in depth was created and filled with saline prewarmed to 30°C to 35°C. The condom tip was positioned 1 cm above the arterial thrombus. Because fluid was absorbed by the surrounding tissue and the fluid level gradually fell during the experiment, saline was added as necessary to maintain an adequate fluid level in the pouch.

For ultrasound-treated (+US) rabbits, continuous wave ultrasound (100% duty cycle) was turned on for 60 minutes (see below) with only brief (a few seconds) interruptions at 5-minute intervals to take blood flow measurements.

The acoustic field resulting from the ultrasonic instrument was measured by placing the transducer face directly under the surface of a water tank, with a needle-type hydrophone (Medicoteknisk Institute, Brondby, Denmark; calibrated against an NBS standard at Elscint, Ltd, Haifa, Israel) submerged in the tank. The spatial peak time average intensity was measured at several distances between 100 and 170 mm. The beam profile was plotted at three axial distances: 100, 135, and 170 mm from the transducer face. Readings were taken at a 15-cm axial distance from the transducer face to simulate the distance between the transducer and clots in the rabbit protocol. All measurements were performed at Elscint Ltd, Haifa, Israel.

Temperature Measurements

The saline temperature in the tented pouch was measured continuously with a mercury thermometer and recorded every 5 minutes in all rabbits. Whenever the temperature rose above 40°C, room temperature saline was added to prevent further heating. As mentioned, saline at room temperature was also added to maintain the fluid level in the pouch, and this also helped to prevent excessive temperature elevation.

For tissue temperature measurements, a laboratory thermistor (Yellow Spring Instrument) was placed on a needle probe and inserted immediately adjacent to, and in contact with, the arterial segment containing the clot. This measurement was performed in eight rabbits—four each from the +US and −US groups. Thermistor insertion and tissue temperature measurements were undertaken at 15-minute intervals, with the ultrasound turned off briefly for measurements, and the thermistor was removed between readings.
Study Protocol

Immediately after confirmation of a stable occlusion, 27 rabbits were randomized into four treatment groups (Table 1) with continuous wave ultrasound on or off, with or without intravenous injection of 17 mg/kg aspirin (Lysozym, Rafa, Ltd, Jerusalem, Israel) (+US/-US/+Asp/-Asp: four combination groups). The first 17 rabbits were randomized into group A (ultrasound off, no aspirin: -US/-Asp) or group B (ultrasound on, no aspirin: +US/-Asp). The final 10 rabbits were randomized into group C (ultrasound off, with aspirin: -US/+Asp) or group D (ultrasound on, with aspirin: +US/+Asp). In groups C and D, the intravenous aspirin was injected as a bolus before recombinant tissue-type plasminogen activator (rTPA) infusion. The rTPA (Genentech, Inc, San Francisco, Calif) was infused at 30 μg·kg⁻¹·min⁻¹ via the marginal ear vein for 60 minutes using a constant rate infusion pump (Harvard Apparatus). Femoral flow was measured every 5 minutes for 2 hours.

Partial recanalization was predefined as blood flow return to 15% to 50% of the stenotic flow value as measured by the flowmeter. Complete recanalization was defined as blood flow return of more than 50% of the stenotic flow values. Recocclusion was defined as flow deceleration to less than 15% of the stenotic flow after recanalization of the artery. The flow values were independently determined by two observers throughout the experiment.

At the end of the experiment, femoral artery patency status was categorized into one of three classes as follows. (1) Persistent occlusion (PO) consisted of no blood flow return (<15% of stenotic flow) throughout 120 minutes of the observation period. (2) Recocclusion after reflow (RR) consisted of recocclusion persisting for at least 30 minutes before the end of the observation period. (3) Persistent patency (PP) consisted of persistent flow without recocclusion for at least 30 minutes before the end of the observation period.

In cases in which changes in arterial patency status occurred more than 90 minutes after starting the rTPA infusion, the observation period was extended from the routine 120 minutes to 150 minutes to verify the stability of the final patency status for categorization purposes into one of the three patency status groups.

Reflow time was defined as the time interval between starting the rTPA infusion and the occurrence of initial recanalization (>15% of the stenotic flow value).

At the end of the experiment, animals were killed by injecting an overdose of pentobarbital.

Statistical Analysis

All statistical analyses were performed using SAS software. Results are expressed as mean±1 SEM unless otherwise indicated. ANOVA was used to compare recanalization times between experimental groups, and the significance of differences between groups was determined using the t test for two unpaired values and the Bonferroni t test for more than two variables. A Kruskal-Wallis nonparametric ANOVA was calculated on ranks of the ordered variables of arterial patency, which ranged from 1 to 3 (1, persistent occlusion; 2, recocclusion after reflow; 3, persistent patency). Fisher's exact test was used to compare the occurrence of reflow and recocclusion between two combined groups allocated to ultrasound on or off. Repeated-measures ANOVA was used to test whether flow and temperature measurements varied among the treatment groups across all time points. The significance of differences at each time period was compared by Duncan’s multiple-range test for variable means. Differences were considered significant when P<.05.

Results

Ultrasonic Transducer Calibration

The water tank measurements by hydrophone yielded a spatial peak time average acoustic intensity of 6.3 W/cm² at 15 cm axially from the transducer, with an accuracy of ±30%. No significant changes in acoustic intensity were found due to axial motion within the distance range of 9 to 20 cm between the transducer and the hydrophone. The measured beam width was more than 1 cm on each side of the insonified segment of artery. This wide beam width was confirmed by lateral hydrophone motion at depths of 10, 13, and 17 cm.

Temperature Measurements

Maximal temperature rises of 7.5±1°C and 1.5±0.5°C were measured by the thermistor in the saline bath in the +US and -US groups, respectively (P<.01) (Fig 2, top). Maximal tissue temperature rises measured by the thermistor adjacent to the thrombosed artery were 7.1±1°C and 2.0±0.5°C in the +US and -US groups, respectively (P<.01) (Fig 2, bottom).

Arterial Flow Measurements

Femoral artery thrombosis was induced in 27 rabbits. The mean weight did not differ among the four treatment groups, nor did mean arterial flow values measured before thrombosis was induced (Table 1).

Arterial patency during the observation period of each individual experiment is shown in Fig 3 for the four treatment groups. Reperfusion persisting to the end of the 120-minute observation period occurred in seven of eight group A (-US/-Asp) and five of five group C (-US/+Asp) rabbits (Table 2). In five of nine group B (+US/-Asp) and four of five group D (+US/+Asp) rabbits, reflow was achieved, but persistent recocclusion subsequently developed in five of five and two of four rabbits, respectively. Overall arterial patency was worse and recocclusion occurred more often with ultrasound exposure (P=.002 by Kruskal-Wallis nonparametric ANOVA).

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Weight, kg*</th>
<th>Stenotic Flow, mL/min*</th>
<th>rTPA</th>
<th>Aspirin</th>
<th>Ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>4.0±0.1</td>
<td>9.1±1.1</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>4.2±0.1</td>
<td>8.8±0.6</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>4.4±0.3</td>
<td>7.2±0.7</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>4.3±0.2</td>
<td>10.5±0.7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

rTPA indicates recombinant tissue-type plasminogen activator.

*Data are given as mean±1 SEM. The differences among the four groups in weight (P>.2) and stenotic flow (P=.13) were not significant by ANOVA.
There were 2 of 14 persistent recanalizations among +US rabbits compared with 12 of 13 -US counterparts (P<.001). Persistent reocclusion was observed more often among +US than -US rabbits (7 of 9 versus none of 12, P<.001, respectively).

Separate analysis of rabbits achieving reflow in each group demonstrates that ultrasound exposure was associated with more limited recanalization as evidenced by plotting separately the mean percentage of stenotic flow against observation time for the four treatment groups (P<.02 by repeated-measures ANOVA) (Fig 4). However, in rabbits achieving initial reflow (Fig 5), it occurred more rapidly with ultrasound (21±10 minutes for group D, +US/+Asp; 33±6 minutes for the group B, +US/-Asp group) than without ultrasound (46±13 minutes for group C, -US/+Asp; 74±14 minutes for group D, -US/-Asp group) (P=.03). Ultrasound exposure was associated with accelerated time to initial recanalization by analyzing the two combined +US (n=9) and -US (n=12) groups sustaining initial recanalization. Recanalization time was 28±6 minutes for the +US combined group compared with 62±10 minutes for their -US counterparts (P<.02).

Discussion

This study was designed to determine the ultrasound effect on thrombolytic results in a preestablished rabbit model. The main finding of our study was that ultrasound at 1 MHz and 6.3 W/cm² significantly shortened the time to initial reflow, but it was associated with worse overall arterial patency and more frequent arterial reocclusion after initial recanalization. The time to flow restoration was synergistically shortened by both ultrasound and administration of aspirin before rTPA infusion.

Addition of Aspirin

Aspirin was added to the protocol in the last 10 animals—groups C and D—because our initial results suggested that ultrasound augments the incidence of reocclusion and raised the possibility of platelet activation by ultrasound, which might counterbalance its thrombolysis-accelerating effect. Aspirin administration was therefore aimed at counteracting this latter effect. Ultrasound has been reported to activate platelets in vitro with a greater effect at lower frequencies (no effect at 3 MHz, little effect at 1.5 MHz, and considerable effect at 0.75 MHz) and higher intensities (at a frequency of 0.75 MHz, little or no platelet activation effect at 2 W/cm², starting effect at 3 W/cm², significantly more effect at 3.4 W/cm², and maximal effect at 4.5 W/cm²). It is therefore hypothesized that platelet activation might explain our results regarding less recanalization and more reocclusion because platelet activation plays a key role in the etiology of thrombolysis failure.

In vitro studies by Chater and Williams and Williams et al also suggest that at the frequency and intensity used in the present study, aspirin may not abolish the ability of ultrasound to cause platelet aggregation, but a lower intensity of ultrasound—approximately 3 W/cm²—might avoid platelet activation. However, ultrasound exposures in that in vitro study were only for 5 minutes.

Comparison of the Present Study With Prior Literature on Ultrasound Effects on Thrombolysis

The finding of a decreased recanalization rate and more reocclusion with external ultrasound application in vivo appears to be contradictory to previous animal studies. In most of these studies, the time to reflow achievement was the only end point examined: these studies were not designed to examine the overall patency rate and the ultrasound effect on spontaneous reocclusion in vivo. The present study confirms previous in vivo studies as well as in vitro experiments by showing the ultrasound-accelerating effect on initial flow restoration and clot dissolution. Other studies have used higher, “ultrasound angioplasty” intensities to achieve direct clot disruption without thrombolytic agents. However, our findings regarding reocclusion contradict those of Yoshizawa, who suggested that ultrasound actually prevented reocclusion in the canine femoral artery thrombosis model. These conflicting results might reflect the differences between the mode of thrombus and reocclusion induction as well as the different ultrasound intensities that were used in the two experiments. Yoshizawa used a balloon catheter for bilateral femoral arterial injury, ligated proximal to the injured segments for a 2-hour period; he then used rTPA to recanalize both femorals and then religated both femorals for 2 hours and applied 200 kHz ultrasound to one femoral artery. He found seven of eight femorals exposed to ultrasound were patent at the end.
of the second 2-hour period, whereas none of eight of the femorals not exposed to ultrasound were patent at the end of the second 2-hour period. This is the only other investigation we are aware of with data on reocclusion rates after ultrasound and thrombolysis.

Because Yoshizawa's protocol is so different from ours, the two studies are difficult to compare. It should be noted that both experimental results are limited to the specific conditions of the experiment—they cannot be extrapolated to the human condition.

Ultrasound variables of frequency and intensity may have varied effects on each of the determinants of thrombolytic reperfusion, reocclusion, and long-term arterial patency. As such, this study does not refute or even contradict prior studies, but it does indicate that future studies must be directed to the in vivo exploration of the effects of ultrasound at varying frequencies and varying intensities on platelet aggregation, recanalization, and rethrombosis.

Table 2. Femoral Artery Patency Status Among the Four Rabbit Treatment Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Persistent Occlusion</th>
<th>Reflow-Reocclusion</th>
<th>Persistent Patency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The difference between the overall patency status was significantly greater for ultrasound off ($P=.002$ by Kruskal-Wallis nonparametric ANOVA—see text for details).

Mechanisms

The mechanism by which ultrasound might accelerate thrombolysis is unclear. Most currently understood ultrasound bioeffects are caused by mechanisms such as heating, acoustic cavitation, and microstreaming.$^{31-33}$ Several studies have suggested that heating is unlikely to be the major factor explaining ultrasound-accelerated thrombolysis,$^{10,11,15,16}$ and one recent study suggests that
it is.\textsuperscript{17} Another proposed mechanism of ultrasound-accelerated thrombolysis based on recent in vitro observations is stable cavitation.\textsuperscript{10,11} Our data do not differentiate among the possible mechanisms because the purpose of the present study was to investigate potential ultrasound efficacy, not to determine its mechanism of action. The mechanism of the effect on reocclusion is unknown because it has not been reported. Potential mechanisms include platelet activation, endothelial changes, or other, unknown, mechanisms.

\section*{Conclusions}

The present study confirms the existence of an ultrasound-accelerating effect on thrombolysis in vivo, but notes a hazard of decreased reperfusion success and early thrombotic reocclusion resulting from ultrasound at the intensity and frequency used in our study. A possible explanation for these results is ultrasound-induced platelet activation counterbalancing the thrombolysis-accelerating effect of ultrasound.

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\section*{References}

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