Ultrasound-Assisted Thrombolysis for Stroke Therapy
Better Thrombus Break-Up With Bubbles

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Background and Purpose—Ultrasound has been shown to increase recombinant tissue plasminogen activator thrombolysis through stable cavitation, or sustained bubble activity, but this mechanism needs further optimization. Use of low-frequency ultrasound in combination with microbubbles stabilized against dissolution, in the form of ultrasound contrast agents, has resulted in greater lytic efficacy in vitro.

Summary of Review—This article reviews the motivation for developing ultrasound-enhanced thrombolysis and the existing evidence for its potential as an intervention for ischemic stroke. Stable cavitation is discussed and current in vitro and ex vivo studies of bubble-mediated recombinant tissue plasminogen activator clot lysis are summarized.

Conclusions—Ultrasound-driven stable cavitation nucleated by an infusion of an echo contrast agent facilitates recombinant tissue plasminogen activator thrombolysis. Optimization of this gently effervescent phenomenon has the potential to reduce the morbidity and mortality of victims of ischemic stroke. (Stroke. 2010;41[suppl 1]:S50-S53.)

Key Words: bubbles ▪ cavitation ▪ rtPA ▪ thrombolysis ▪ ultrasound

The administration of recombinant tissue plasminogen activator (rtPA) in patients with ischemic stroke is only moderately effective and results in a 30% greater chance of little or no disability compared with placebo at 3 months.1 A 6.4% incidence of intracerebral hemorrhage exists in patients receiving this thrombolytic therapy.1 Thus, an improved intervention that could increase lytic efficacy and reduce the incidence of intracerebral hemorrhage would lead to a significant improvement in outcomes for patients with stroke.

Adjuvant ultrasound has been shown to enhance rtPA thrombolysis in vitro and in vivo.2-8 Improvement of lysis locally using an ultrasound beam may enhance the efficacy and has the potential to improve the safety of rtPA treatment.

In vitro studies have shown that ultrasound-assisted thrombolysis is mediated by stable cavitation, or sustained bubble activity.9-11 Acoustic cavitation has been studied extensively both theoretically and experimentally by investigators in an attempt to understand the cause of ultrasound bioeffects.12-22 Acoustic cavitation is the formation and collapse of gaseous and vapor bubbles in a liquid due to an acoustic pressure field. Cavitation is generally classified into 2 types: stable cavitation, which results in emissions at the subharmonic and odd ultraharmonics of the main excitation frequency, and inertial cavitation, which is characterized by broadband noise emissions. When a bubble oscillates nonlinearly about its equilibrium radius, Faraday waves are excited along the bubble wall causing subharmonic emissions.23 Broadband noise emissions are generated when bubbles undergo large radial oscillations and collapse violently.15,16,24,25 This type of bubble motion is dominated by the inertia of the surrounding fluid, hence the label “inertial” cavitation. Stable cavitation can induce microstreaming26 and inertial cavitation can cause microjetting and pitting on solid surfaces.27-29

Enhancement of rtPA Thrombolysis With Low-Frequency Ultrasound

Low-frequency ultrasound (<1 MHz) is a good choice for skull penetration30 and accelerates rtPA thrombolysis.31-33 Alexandrov et al34 used 2-MHz transcranial Doppler ultrasound to monitor flow in the middle cerebral artery in patients with acute ischemic stroke and observed an increase in the rate of sustained complete recanalization within 2 hours after the administration of rtPA in those patients who were monitored with ultrasound. Unfortunately, another clinical trial using transcranial 300-kHz ultrasound, the TRanscranial low-frequency Ultrasound-Mediated thrombolysis in Brain Ischemia (TRUMBI) trial, was ended early when 13 of 14 patients being treated with rtPA and ultrasound had evident intracranial bleeding on MRI compared with 5 of 12 treated with rtPA alone and 5 of them experienced symptomatic hemorrhage within 3 days of treatment.35 Later simulations showed that standing waves produced inside the skull during this trial may have produced peak rarefractional pressures in excess of 1 MPa,36 and exploration of treatments using lower-amplitude insonation are warranted.
Injection of Definity, a Food and Drug Administration-approved ultrasound contrast agent, allows blood plasma to become a good substrate for stable cavitation when exposed to low ultrasound pressure amplitudes. Ultraharmonic emissions can be used to monitor and measure cavitation activity, and such signals are significantly correlated with increased rtPA lytic efficacy in vitro. The synergistic effect of sustained bubble activity has also been observed at a significantly lower concentration of rtPA (1.4 μg/mL), thus providing the potential for increased safety profile of this drug clinically.

Molina et al combined a galactose-based ultrasound contrast agent and rtPA with 2-MHz transcranial Doppler in patients with acute ischemic stroke. Control subjects received rtPA alone, and a third group received ultrasound and rtPA without the ultrasound contrast agent. The recanalization rate at 2 hours was significantly greater in those who received combined rtPA, ultrasound, and ultrasound contrast agent treatment (54%) compared with rtPA and ultrasound (41%) and rtPA alone (24%). The long-term outcomes of each treatment were not studied. Overall, ultrasound-enhanced thrombolysis is a promising therapy for stroke, but much work remains to be done to demonstrate its clinical advantage.

**Bubble-Driven Thrombolysis in an Ex Vivo Artery Flow System**

An ex vivo artery model that incorporates physiological flow and pressure has been developed to study cavitation-mediated drug delivery and thrombolysis. Aged porcine whole blood clots were placed into the lumina of living, excised porcine carotid arteries to simulate the human middle cerebral artery. Pooled, citrated porcine plasma flowed slowly through the artery, (0 to 8 mL/min, mean 2.7±1.8 mL/min) to simulate the reduced flow environment during ischemic stroke. Each clot was weighed before and after its 30-minute treatment to provide clot mass loss as a metric of thrombolytic efficacy. Combinations of the following treatments were applied: rtPA (3.9±2.1 μg/mL), Definity microbubbles (0.38±0.21 μL/mL), and ultrasound (120-kHz continuous wave at 0.44 MPa peak-to-peak pressure amplitude). The ultrasound was used in an intermittent scheme so that there was periodic replenishment of Definity microbubbles in the volume of plasma surrounding the intraluminal blood clot.

Because flow in the middle cerebral artery in ischemic stroke is very low, if present, the quiescent periods were 19.5 seconds to facilitate Definity influx between continuous-wave ultrasound episodes lasting 8.5 seconds each. Cavitation activity was monitored throughout each treatment.

In the ex vivo flow system, porcine plasma alone (carrying 8 ng/mL of endogenous tPA) produced a clot mass loss of 12% over a 30-minute sham treatment, as shown in Figure 1. The addition of rtPA resulted in 29% clot mass loss. Com-
bination of Definity with rtPA, or ultrasound with rtPA and no Definity, did not increase mass loss over rtPA alone. However, when Definity and ultrasound were combined to produce stable cavitation in the presence of rtPA, mass loss increased to 83%. The maximum mass loss achieved was limited by the physical arrangement because only two thirds of the clot length sat within the beam of the ultrasound. No enhancement of rtPA extravasation was noted in any of the arteries by a pathologist blinded to the exposure conditions, as shown in Figure 2.

Future Studies
A targeted thrombolytic delivery system using echogenic liposomes is under development. Studies in vivo have demonstrated successful targeting of the echogenic liposomes to a thrombus surface using inactivated tPA. An in vitro study demonstrated effective use of selectively tuned clinical diagnostic ultrasound to release drug from rtPA-loaded echogenic liposomes. The rtPA-loaded echogenic liposomes will be tested in the ex vivo artery system to determine optimal ultrasound parameters for delivery of the lytic drug and for promotion of stable cavitation to enhance efficacy. The encapsulation of a lytic combined with a cavitation nucleation agent and gas, which promotes vasodilatation within a lipid bilayer, has the potential to restore physiological flow with minimal exposure of the whole body to the enzyme.

Conclusions
A new treatment that limits the whole-body dose of rtPA at the same time as maintaining its efficacy in a spatially controlled fashion could reduce the hemorrhagic complications of lytic treatment. Adjunctive use of ultrasound-driven bubble activity during intravenous rtPA treatment has the potential to enhance thrombolytic therapy. A number of stabilized microbubble preparations, some of which are already approved by the Food and Drug Administration as ultrasound contrast agents, make it possible to use low-ultrasound pressure amplitudes to promote stable cavitation, thereby reducing the potential for negative ultrasonic bioeffects. Ongoing in vitro, ex vivo, and in vivo studies are aimed at developing this promising technique.

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Disclosures
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