Ultrasound Enhancement of Fibrinolysis
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Abstract—Systemic administration of tissue plasminogen activator (tPA) remains the fastest way to initiate treatment for acute ischemic stroke. The presence of a proximal arterial occlusion should not be viewed as an insurmountable predictor of tPA failure. Because tPA works by induction of partial recanalization of large thrombi, early augmentation of fibrinolysis to improve recanalization is desirable. This augmentation is feasible and can be safely achieved at the bedside with diagnostic Doppler ultrasound. In the CLOTBUST trial, 83% of patients achieved any recanalization (46% complete, 27% partial) with tPA+transcranial Doppler vs 50% (17% complete, 33% partial) with tPA alone within 2 hours of treatment ($P<0.001$). Sustained, complete recanalization at 2 hours was $38\%$ vs $13\%$, respectively ($P=0.03$).

A recent meta-analysis of 6 randomized and 3 nonrandomized clinical studies of sonothrombolysis showed that any diagnostic ultrasound monitoring can at least double the chance of early complete arterial recanalization at no increase in the risk of symptomatic intracerebral hemorrhage. Because application in humans of frequencies below the diagnostic range resulted in increased symptomatic bleeding rates, mechanisms by which megahertz and kilohertz frequencies interact with the clot–residual flow interface and endothelium are currently under renewed investigations. Catheter-based ultrasound delivery to arterial thrombi and intraventricular clots is the subject of ongoing clinical trials. Addition of gaseous perflutren-lipid microspheres to tPA and transcranial Doppler can further facilitate early flow improvement, with a 50% rate of early, complete recanalization in a recent feasibility study. Transcranial ultrasound delivery in an operator-independent and dose-controlled manner is being tested in a clinical trial. (Stroke. 2009;40[ suppl 1]:S107-S110.)

Key Words: tPA ■ ultrasound ■ stroke ■ thrombolysis ■ outcomes

Systemic administration of tissue plasminogen activator (tPA) remains the only approved therapy for acute ischemic stroke. The intravenous route also remains the fastest way to initiate treatment, particularly at the primary stroke center level. The presence of a proximal arterial occlusion should not be viewed as an insurmountable predictor of tPA failure because nutritive recanalization can occur even with large middle cerebral (MCA) or internal carotid artery thrombi. Even if intra-arterial rescue is approved in the future for stroke treatment, it is unrealistic to expect that all patients with MCA occlusions either will reach comprehensive stroke centers in time or their risk factor profile would always make catheter intervention feasible. With bridging intravenous–intra-arterial protocols being tested, there is even further need to amplify the systemic part of reperfusion therapy so that more patients could benefit from early treatment initiation.

Because intravenous tPA by itself works by induction of mostly partial recanalization of large thrombi, early augmentation of fibrinolysis to improve arterial recanalization is desirable. Fibrin strands are cross-linked and held by activated platelets to form a structure that looks like a fisherman’s net that captures red blood cells (Figure 1). To capture red blood cells, the net should have openings of $<$6 μm (note that the average size of erythrocytes is 6 to 8 μm). By analogy with fishing, it is designed to capture large “fish” that are important, ie, to prevent erythrocytes escaping from the bloodstream if bleeding occurs. In the case of ischemic stroke, thromboembolic material lodges at the wrong time in a wrong place. The thrombus size and structure will further create an area of high resistance to antegrade movement of red blood cells, and once the embolus reaches a vessel comparable to its size, it further acts like a plug, thus precluding flow if arterial pressures are insufficient to further distend the vessel wall and maintain residual flow adequate to sustain neuronal function just distal to the acute occlusion. Given these circumstances, delivery of tPA to and through the thrombus is likely dependent on minuscule residual red blood cell flow around the thrombus and plasma flow through the thrombus. Mechanical agitation can expose shallow layers of thrombi to circulating tPA and facilitate streaming of plasma through the thrombus, thus bringing more tPA to binding sites. Ultrasound is a pressure wave that can travel through tissues and deliver this mechanical momentum to stagnant flow areas and clot interfaces. Numerous experiments have confirmed the ability of ultrasound to enhance fibrinolysis through disaggregation of non–cross-linked fibrin strands, further thinning them, and promoting plasma flow and lytic drug delivery to the clot. Kilohertz frequencies were thought to induce more mechanical stretching, whereas megahertz ultrasound promotes fibrinolysis more through enzymatic mechanisms.

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Independent of this basic science research, our group serendipitously discovered as part of the clinical routine that stroke patients treated with systemic tPA and monitored with 2-MHz transcranial Doppler (TCD) could recanalize early during treatment and experienced dramatic clinical recovery.5 Our group further has shown the feasibility and safety of thrombolysis monitoring and augmentation at the bedside with TCD.5,7 These results were confirmed by other groups worldwide who monitored tPA infusion by diagnostic ultrasound.13,14

We formed a multicenter collaborative group to test this observation in a clinical trial. In the Combined Lysis of Thrombus in Brain ischemia using transcranial Ultrasound and Systemic tPA (CLOTBUST) trial,7 83% of patients achieved any recanalization (46% complete, 27% partial) with tPA and TCD versus 50% (17% complete, 33% partial) with tPA alone within 2 hours of treatment (P<0.001). Sustained complete recanalization at 2 hours was 38% versus 13%, respectively (P=0.03). The symptomatic intracerebral hemorrhage rate was 3.8% in both groups (P=NS).7 This trial was the first properly powered clinical trial that confirmed the existence of ultrasound-enhanced thrombolysis in human subjects and demonstrated a positive biologic effect of diagnostic, low-power ultrasound.

A recent meta-analysis of 6 randomized and 3 nonrandomized clinical studies of sonothrombolysis (currently published as an abstract) suggested that any diagnostic ultrasound monitoring can at least double the chance of early complete arterial recanalization at no increase in the risk of symptomatic intracerebral hemorrhage.15 Ultrasound can be delivered to an acute arterial occlusion in several ways: (1) by an experienced sonographer who detects spectral Doppler residual flow waveforms, called the Thrombolyis In Brain Ischemia system,16 or B-mode/color flow duplex imaging;14,17 (2) an operator-independent externally applied system that delivers diagnostic levels of ultrasound exposure to tissues;18 (3) unfocused, low-frequency ultrasound that sonicates both the vessels and brain without any imaging;19 (4) high-intensity, focused ultrasound with magnetic resonance imaging guidance;20 and (5) intra-arterial or intraclot delivery via catheter, such as the EKOS technology.21

Precision in ultrasound delivery to the thrombus can be achieved with refinement of operator-independent ultrasound targeting,18 imaging technologies,22–25 contrast microspheres tagged to recognize and adhere to the thrombus,22 crossed beams under magnetic resonance imaging guidance,20 and direct embedding into the clot.21 Some of these technologies are in the first experimental stages and some are already in clinical trials. Since application in humans of frequencies below the diagnostic range (ie, kilohertz) resulted in increased symptomatic bleeding rates,19 while 2 MHz showed a strong sign of efficacy and safety,7 the mechanisms by which kilohertz and megahertz frequencies interact with the clot–residual flow interface, endotheium, and brain tissues are currently under renewed investigations.26–28 Catheter-based ultrasound delivery to arterial thrombi and intraventricular clots is the subject of ongoing clinical trials29 (D.N. Newell, personal communication, 2008).

Among these diverse investigations conducted by numerous research groups worldwide, our multicenter collaborative group continues prospective studies and clinical trials in the use of safe TCD technology and methods of the CLOTBUST trial.30 Molina et al31 pioneered the use of gaseous microspheres in combination with CLOTBUST monitoring methods and reported safety and recanalization rates with first-generation, air-filled microspheres (Leovist), as well as with newer diagnostic microspheres (Sonovue, Bracco).32 Note that these microspheres were designed for diagnostic purposes to be given as boluses to enhance returned echoes and improve the quality of ultrasound images.
However, regardless of the type of microspheres, they share the same mechanisms by which they respond to the ultrasound pressure wave. After intravenous injection, microspheres circulate in the bloodstream and cross the lung capillaries. When intercepted by an ultrasound beam, these microspheres undergo expansion in size, followed by a transient oscillation or at times complete breakup (Figure 2A). The likelihood of oscillation or cavitation with microsphere destruction may depend not only on the resonant frequency but also on the power of ultrasound delivered to the target tissues. These processes are perhaps better described as microspheres’ fragmentation while fusion may also be possible under variety of ultrasound exposure levels (K. Ferrara, personal communication, 2008).

In our in vitro experiments with the temporal bone/MCA flow model, we observed that even low-power and bone-attenuated TCD can decrease the count of microspheres that passed through a small diagnostic ultrasound beam. We also confirmed that reflections, even from a single, oscillating microsphere is possible to be traced by conventional TCD equipment. When microspheres oscillate or burst, they expand like microscopic balloons, move in 3 dimensions, and transmit mechanical energy momentum from the passing ultrasound wave to the surrounding fluid and structures. If an ultrasound beam intercepts microspheres at the clot–residual flow interface, this facilitates fibrinolysis with or even without tPA. Our group has further explored the therapeutic use of the newest generation of microspheres, such as perfluorinated lipid microspheres that are more stable in solution (they tend to “bubble up” less than their predecessors) and therefore are suitable for continuous infusion. This in turn enables continuous replenishment of microspheres at the clot–residual flow interface during the entire tPA infusion. Our pilot multicenter feasibility study showed that perfluorinated lipid microspheres reached intracranial thrombi in all patients, and in 75% they immediately permeated through or around the thrombi and reached areas with no detectable flow (Figure 2B). The first dose of these microspheres was safely coadministered during tPA infusion in the contralateral arm, causing no symptomatic intracerebral hemorrhage. As a sign of efficacy, perfluorinated lipid microspheres, TCD, and tPA lysed 50% of proximal MCA occlusions, a figure that compares favorably with both concurrent and historic control subjects who received tPA alone. Another multicenter microsphere dose-escalation study, Transcranial Ultrasound in Clinical SNOthrombolysis (TUCSON; NCT00504842) was recently completed, and the results are pending presentation at the
next stroke conference. Transcranial ultrasound delivery in an operator-independent and dose-controlled manner is now being tested in a separate clinical trial by the University of Alabama and the University of Texas-Houston stroke teams.

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Disclosures
Dr Alexandrov reports having served as a consultant to ImaRx Therapeutics, Inc. Dr Alexandrov holds a US patent, “therapeutic agents and apparatus for use of sonication to enhance perfusion of tissue” (6,733,450), and he is a founder of Vitason Technologies. Dr Alexandrov reports having served as a consultant to ImaRx Therapeutics, Inc. Dr Alexandrov holds a US patent, “therapeutic...
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