In 1974, Sobbe et al.1 applied 26.5-kHz ultrasound (US) to recanalize thrombosed iliofemoral arteries in dogs with minimal complications. These pioneer efforts were followed by studies showing that catheter-based or transcutaneous US can enhance the effect of fibrinolytic agents in recanalizing thrombosed arteries,2–8 thus paving the way for first clinical studies evaluating the adjunct effect of US in treating patients with ischemic stroke.

Mechanisms

US Thrombolysis

Despite numerous studies documenting a thrombolytic effect of US, the mechanisms remain poorly understood. Inertial cavitation (ie, the formation and violent collapse of gas-filled bubbles in a fluid exposed to US) gave rise to transient microjets that disintegrate thrombus mechanically.9 Stable cavitation (ie, sustainable nonlinear periodic contraction or expansion of a gas body or bubble) may be more effective than inertial cavitation in clot lysis.10 US also facilitates penetration of fibrinolytic drugs into the thrombus and binding to fibrin.11 This is because US promotes the motion of fluids around the clot surface through a process called microstreaming. Moreover, pressure waves may increase the permeation of tissue-type plasminogen activator (tPA) into the interior of the fibrin network.12 Heating is uniformly present in tissue exposed to US but has been deemed too mild to explain thrombolytic effects.

Microbubble-Enhanced Thrombolysis With tPA

Significant amplification of lysis occurs with the addition of microbubbles to the combination of thrombolytic drug and US.13–15 Microbubbles, composed of lipid, albumin, or galactose shells and ranging in size from 0.5 to 5 μm, lower the threshold for thrombolysis by providing a pre-existing bubble that easily can be made to cavitate by US. Stable cavitation can produce microstreaming in the area and dramatically enlarge the bubble momentarily. This will cause localized mechanical stress on the adjacent clot. The surface of the clot will erode, and even penetration and numerous microscopic holes inside the clot have been demonstrated.9 Microstreaming also leads to a dramatic increase in delivery of thrombolytic drug to the clot.16 More energy delivered to the microbubble can lead to inertial cavitation, which ends with violent disruption of the bubble. This can produce microjets that are also effective in eroding clot.17 Pressure waves interact with bubbles, causing expansion and contraction. This can lead to “pumping” of energy from the traveling wave, “focusing,” and re-emitting it locally, sometimes at other, perhaps better frequencies.

Clot Lysis With US and Microbubbles Without Thrombolytic Drugs

Thrombolysis without thrombolytic drugs can be readily accomplished with microbubbles and US. In vitro and in vivo studies have confirmed this on several scales ranging from tiny to large clots.10,15,18–23 In vitro examples show a predominantly mechanical effect of the bubble encountering the clot and eroding it when activated by US.10,15,19 In vivo examples are almost certainly more complex with interactions also including endothelial factors triggered by local ischemia as well as an endogenous tPA effect originating from the ischemic vessel wall.18,20–23 Recently, treatment with microbubbles and US in a rabbit model of embolic stroke was shown to decrease the incidence of intracerebral hemorrhage24 as compared with tPA and also to result in similar reduction of infarct volume as tPA.25

Targeted Sonothrombolysis

Albumin microbubbles have been tagged with the glycoprotein IIb/IIIa inhibitor eptifibatide to promote their accumulation at the clot for enhanced recanalization.22 Likewise, abciximab microbubbles targeted to human platelets improve visualization of human clots both in vitro and in an in vivo model of acute arterial thrombotic occlusion, thus demonstrating the feasibility of using a therapeutic agent for selective targeting in vascular imaging.26 Importantly, ligand targeting of bubbles with abciximab improves the effectiveness of lysis with US.27

Entrapment of tPA into liposomes can also improve the efficacy of thrombolysis28–33 through cavitation effects and acoustic radiation force.34 Recent work suggests that tPA-loaded echogenic liposomes are superior to microbubbles for
clot lysis with US.\textsuperscript{35} Novel developments combine nanotechnology with microbubbles for drug delivery.\textsuperscript{36}

**Sonothrombolysis Through the Human Skull**

Different US equipment has been proposed for performing sonothrombolysis in patients with stroke, each with its advantages and disadvantages. One avenue has been to apply commercial 2-MHz transcranial monitoring devices to accelerate clot lysis with recombinant tPA. Although this choice fosters rapid translation of sonothrombolysis into the clinical arena because of existing device approvals, some work has questioned whether this approach is really capable of treating ischemic stroke because of the very high attenuation of US by temporal bone.\textsuperscript{37} In humans this amounts to a reduction of at least 86% of the US energy of diagnostic transducers in very thin bone windows\textsuperscript{87} and to almost 100% in patients with poor bone windows. Differences in signal absorption of the bone can lead to 10-fold differences in the mechanical index of the incident US wave.\textsuperscript{38} The skull also greatly distorts the US field of commercial diagnostic frequencies, which causes changes in the beam area by a factor of approximately 4 (ie, defocusing through phase aberration).\textsuperscript{39} Thus, both the local “effective” US acoustic pressure and the treatment area delivered to any 1 patient for thrombolysis vary significantly, which makes comparisons of therapeutic efficacy between patients difficult. Recent computer simulations suggest that intracranial acoustic pressures achieved with diagnostic devices are not high enough to enable enhancement of tPA thrombolysis.\textsuperscript{39} One may argue, however, that the thrombolytic effect in living systems is far more complex and may be explained through an enhancing effect of US on endogenous tPA for clot lysis, local renewing of plasminogen, and possible evacuation of dissolved thrombotic material by collaterals. Such hypothetical in vivo mechanisms await further substantiation.

Dedicated equipment using lower US frequencies than diagnostic machines is theoretically better suited for thrombolysis through the skull\textsuperscript{40,41} because of better penetration and superior thrombolytic effects. The use of lower frequencies in the skull, however, is more complex with potential adverse effects not occurring with diagnostic frequencies. These are discussed in detail in relation to the Transcranial Low-Frequency Ultrasound-Mediated Thrombolysis in Brain Ischemia (TRUMBI) trial (see subsequently).

**Clinical Data**

**US-Enhanced Thrombolysis of Ischemic Stroke**

Several groups have reported the use of commercial 2-MHz diagnostic US devices for treating acute ischemic stroke.\textsuperscript{42–44} The largest of these was Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic tPA (CLOTBUST),\textsuperscript{45} a multicenter randomized clinical trial on 126 patients with acute occlusion of the middle cerebral artery. All patients were treated with intravenous recombinant tPA within 3 hours after the onset of symptoms. Target patients received 2-MHz transcranial Doppler monitoring for 2 hours along with recombinant tPA. A complete reperfusion or dramatic clinical recovery was observed for 49% of the patients in the target group (recombinant tPA+US) and for only 30% of the control group. No secondary effects linked with US exposure were identified.

The TRUMBI trial used a dedicated low frequency 3000kHz US device for sonothrombolysis.\textsuperscript{46} The reasons for choosing this frequency were 3-fold: (1) penetration through the skull is superior to commercial 2-MHz probes; (2) the thrombolytic effect is faster and more efficient\textsuperscript{44}; and (3) the approach is simpler because a large volume of the brain is sononated when using low-frequency US, thus ensuring better targeting of occlusions, for example, middle cerebral artery occlusions with a variable anatomic course and branch occlusions not accessible to diagnostic US devices through the temporal bone window. However, the trial was stopped prematurely because of the occurrence of a higher number of intracerebral hemorrhages after tPA treatment combined with transcranial sonication, some occurring remote to the ischemic lesion. Five hemorrhages in the target group were symptomatic, possibly linked to US exposure.

The reason for hemorrhages in the TRUMBI trial was unclear. Indeed, preclinical work in rats\textsuperscript{47} was unable to demonstrate harmful secondary effects of US using the TRUMBI parameters. One study suggests that hemorrhages in TRUMBI were related to abnormal permeability of the human blood–brain barrier that was induced by wide-field low-frequency insonation.\textsuperscript{48} Wang and coworkers hypothesize that in TRUMBI, a pulse length of 765 mm combined with a very wide beam can cause overlap many times as the wave runs its course back and forth across the brain, reflecting off the skull. Therefore, the instantaneous intensity of US in the brain tissue may multiply constructively at some localized sites of brain tissue, resulting in mechanical indexes that are larger than the maximum limit set by the Food and Drug Administration.\textsuperscript{49} A recent simulation of the TRUMBI trial demonstrated that pressure levels in the brain (approximately 0.27 MPa) were just slightly above the inertial cavitation threshold, which could result in standing waves outside the targeted region.\textsuperscript{50} Importantly, these adverse effects could be remedied through small adjustments of the US parameters in the simulation studies.

**Clinical Studies of Microbubble-Enhanced Thrombolysis**

The first clinical trial using microbubbles in acute stroke added Levovist to standard tPA therapy augmented with continuous application of transcranial Doppler.\textsuperscript{50} Outcomes were improved to a 55% sustained recanalization rate compared with 41% using tPA and continuous transcranial Doppler and 24% using tPA alone. Clinical improvement was ≥4 National Institutes of Health Stroke Scale in 55% compared with 41% with tPA and continuous transcranial Doppler and 31% with tPA alone. No increased symptomatic intracranial hemorrhage was encountered.

Another study\textsuperscript{51} reported improved recanalization flow scores and clinical outcomes with a combined microbubble therapy using SonoVue, whereas the use of perflutren lipid microbubbles showed a good safety profile with no increase in symptomatic intracranial hemorrhage after systemic thrombolysis.\textsuperscript{52} In a further pilot investigation, perflutren lipid microbubbles and US were administered with tPA to
patients with proximal intracranial occlusions.\textsuperscript{53} In patients receiving 1.4-mL microbubbles, there were no symptomatic intracranial hemorrhages (ICHs) as opposed to 27\% spontaneous ICHs in patients receiving 2.8 mL. Thus, 2 early-phase studies\textsuperscript{2,53} showed that at the same dose of 1.4 mL of perflutren lipid microbubbles, no symptomatic bleeding events occurred. Lack of knowledge of the effective intracranial acoustic pressures in individual patients makes treatment comparisons in these studies difficult, however.

A recent meta-analysis of all published clinical sonothrombolysis studies (see online-only Data Supplement Table I; http://stroke.ahajournals.org) confirmed that US and tPA (with or without microbubbles) increases recanalization compared with tPA alone.\textsuperscript{54} These observations have led to design of CLOTBUSTER, a Phase III controlled clinical trial of sonothrombolysis (www.clinicaltrials.gov/NCT01098981).

New Developments and Future Directions

Sonothrombolysis of Spontaneous ICHs

Recently, there has been interest in lysis of spontaneous ICH and intraventricular hemorrhage using catheter-mounted transducers. As compared with Minimally Invasive Surgery plus tPA for Intracerebral Hemorrhage Evacuation (MISTIE) and Clot Lysis Evaluating Accelerated Resolution of Intraventricular Hemorrhage II (CLEAR) studies data, the rate of lysis during treatment for intraventricular hemorrhage and ICH was faster in patients treated with sonothrombolysis plus recombinant tPA versus recombinant tPA alone.\textsuperscript{55} Thus, lysis and drainage of spontaneous ICH and intraventricular hemorrhage with a reduction in mass effect can be accomplished rapidly and safely through sonothrombolysis using stereotactically delivered drainage and US catheters through a burr hole.

MRI-Guided Focused US for Clot Lysis

Histotripsy is a process that fractionates soft tissue through controlled cavitation using focused, short, high-intensity US pulses. Histotripsy can be used to achieve effective thrombolysis with US energy alone at peak negative acoustic pressures >6 MPa, breaking down blood clots in approximately 1.5 to 5 minutes into small fragments less than 5 µm in diameter.\textsuperscript{56} Recent developments in using MR-guided focused US therapy through the intact skull suggest that this technology could be useful for clot lysis in humans. Experimental studies are currently being undertaken to test this possibility, both in ischemic and hemorrhagic stroke.

Beneficial Effects of US and Microbubbles on the Microcirculation

The effects of 2-MHz US and microbubbles (Sonovue) have been studied in a middle cerebral artery permanent occlusion model in rats to evaluate possible adverse bioeffects\textsuperscript{57} at different steps in the cascade of tissue destruction after ischemic stroke.\textsuperscript{58} Whereas deleterious effects were not observed, infarctions were unexpectedly smaller in the treatment group despite the fact that in all animals, recanalization of the middle cerebral artery did not occur. This suggested a beneficial effect of US and microbubbles in the microcirculation. A similar tissue protective effect was found in an in vivo animal study using intravenous microbubbles and transient thoracic US to treat acute coronary thromboses. Pigs treated with US and intravenous perfluorocarbon microbubbles had significantly greater improvements in ST segments over a 30-minute treatment period when compared with pigs treated with US alone or with control animals. Moreover, there was a significantly smaller myocardial contrast defect size after treatment with US and intravenous perfluorocarbon microbubbles.\textsuperscript{59} Recently, nano-CT was used to demonstrate complete reversal of microcircular impairment in a rodent reperfusion model after treatment with recombinant tPA, US, and microbubbles.\textsuperscript{60} The mechanism of the microcirculatory effect of US and microbubbles may involve improvement of blood flow to risk tissue through collaterals and changes in the microenvironment of damaged tissue, like decreased cell-damaging factors, for example, glutamate or enhanced enzyme activity of endothelial nitric oxide.\textsuperscript{61} Further work is necessary to elucidate the exact mechanisms of salvaging of tissue at risk by US-mediated microbubble thrombolysis.

Safety of US and Microbubbles in a Model of Intracranial Bleeding

There has been some concern regarding possible capillary ruptures occurring during application of US and microbubbles that might lead to increased bleeding.\textsuperscript{62} Fortunately, this concern has been considerably reduced by work in rabbits showing that current diagnostic US exposure levels combined with microbubbles are well below the threshold of blood–brain barrier opening or brain tissue damage.\textsuperscript{63} One study has investigated whether US and microbubbles influence the course of intracerebral hemorrhage in a rodent model of ICH.\textsuperscript{64} The morphometric evaluation of hemorrhage size and brain edema after application of US and microbubbles showed no significant effect between the treatment and control groups. There was likewise no difference in apoptosis rates. This lack of an effect of US and microbubbles on cerebral hemorrhage provides first experimental support that bleeding may not be a contraindication to treatment of ischemic stroke with this new approach.

Summary

Rapid restoration of vascular flow is the primary goal of acute stroke treatment. US offers new thrombolytic mechanisms when combined with microbubbles. Recent data suggesting that US and microbubbles can improve microvascular flow may provide new concepts for treatment of stroke. Therapy of the microcirculation will require new transducer designs for US delivery to this therapeutic target. Moreover, systems that estimate the intracranial acoustic pressure through direct detection of cavitation or through observations of microbubble behavior will allow more consistent application of US parameters to individual patients in clinical trials.

US-sensitive thrombolytic drug delivery combined with specific targeting is highly attractive. Targeting of clot-dissolving therapeutics could potentially decrease the frequency of complications at the same time as simultaneously increasing treatment effectiveness by concentrating the available drug at the desired site and permitting a lower systemic dose.\textsuperscript{65}
Any new system must be simple and safe when widely used. If safety can be proven even in hemorrhagic strokes, it may well be possible to use some technique of microbubble-augmented US lysis or US-augmented thrombolysis in patients who have not yet been transported to the hospital for CT to determine the presence or absence of intracranial hemorrhage. Even if the improvement in outcomes is only moderate with this new technique, the addition of many more patients to therapy within the earliest part of the time window would be a service with tremendous impact.

**Disclosures**

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