Ultrasound-Enhanced Thrombolysis in Acute Ischemic Stroke: Potential, Failures, and Safety

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Summary: Experimental and pilot clinical evidence shows that thrombolysis with intravenous tissue plasminogen activator (TPA) can be enhanced with ultrasound. Ultrasound delivers mechanical pressure waves to the clot, thus exposing more thrombus surface to circulating drug. The international multicenter phase II CLOTBUST trial showed that, in patients with acute ischemic stroke, transcranial Doppler (TCD) monitoring augments TPA-induced arterial recanalization, with a nonsignificant trend toward an increased rate of recovery from stroke, compared with placebo. In the CLOTBUST trial, the dramatic clinical recovery from stroke coupled with complete recanalization within 2 hours after TPA bolus occurred in 25% of patients treated with TPA + TCD ($n = 63$), compared with 8% of those who received TPA alone ($n = 63$, $P = 0.02$). Different results were achieved in smaller studies that used transcranial color-coded duplex sonography (TCCD) and a nonimaging therapeutic ultrasound system. The findings of the TRUMBI trial (26 patients) underscored the adverse bioeffects of midkilohertz (300 kHz) ultrasound, such as promotion of bleeding in brain areas both affected and unaffected by ischemia. Exposure to multifrequency, multielement duplex ultrasound resulted in a trend toward a higher risk of hemorrhagic transformation. To further enhance the ability of TPA to break up thrombi, current ongoing clinical trials include phase II studies of a single-beam, 2-MHz TCD with perflutren lipid microspheres. Enhancement of intra-arterial TPA delivery is being clinically tested with 1.7–2.1 MHz pulsed-wave ultrasound (EKOS catheter). Multinational dose escalation studies of microspheres and the development of an operator-independent ultrasound device are underway. **Key Words:** Tissue plasminogen activator, transcranial Doppler, stroke, thrombolysis, microspheres.

THROMBOLYSIS IN ACUTE CEREBRAL ISCHEMIA

Unlike thrombolysis for myocardial ischemia, the pilot clinical studies of thrombolysis for ischemic stroke did not document any dramatic Lazarus or on-the-table clinical recovery during treatment.[1–3] Subsequent pivotal trials of tissue plasminogen activator (TPA) have not reported any differences between the groups at 2 and 24 hours post treatment in the prespecified endpoints.[4–7] However, a post hoc analysis of the NINDS trial showed that by 24 hours, 27% of TPA-treated patients improved by $\geq$10 points on the National Institutes of Health Stroke Scale (NIHSS) or resolved their neurologic deficit completely, compared with 12% in the placebo group.[8] Therefore, some patients may have experienced early clinical recovery, presumably due to fast thrombus dissolution, but the overall number of these events was low.

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Early clinical improvement after stroke usually occurs after arterial recanalization.[9–12] A recent meta-analysis confirmed the recanalization hypothesis by showing that recanalization is associated with a four- to fivefold increase in the odds of a good final functional outcome and a four- to fivefold reduction in the odds of death.[13] These results lend strong support to the use of restoration of vessel patency as a surrogate endpoint in phase II trials of neurotherapeutic recanalization agents and in trials comparing novel to existing recanalization devices in acute ischemic stroke. Because early recanalization can lead to dramatic recovery, any additional enhancement of TPA-associated thrombus dissolution can possibly produce even higher early recovery rates among patients with ischemic stroke.

EXPERIMENTAL EVIDENCE FOR ENHANCEMENT OF THROMBOLYSIS WITH ULTRASOUND

The ability of ultrasonic mechanical pressure waves to enhance thrombolysis was first documented in 1976 and 1981,[14,15] and was confirmed by several investigators in

experimental models.[16–20] Various ultrasound energies (0.2–2.0 W/cm^2) and frequencies (20 kHz to 2 MHz) were used in these studies. Ultrasound can promote the motion of fluid around the thrombus, an effect called *microstreaming*.[21] It is possible that the application of ultrasound energy agitates the blood close to the occluding thrombus and promotes the mixing of TPA, effectively increasing the concentration of the agent that is in contact with the thrombus. Consequently, it has been hypothesized that, in stroke patients, ultrasound can promote TPA delivery to the areas with stagnant flow near the occlusion.

The pressure waves generated by ultrasound may also increase the permeation of TPA into the interior of the fibrin network. In addition, ultrasound waves can have direct effects on the binding of TPA to the fibrin mesh that forms the occlusive lesion. Ultrasound energy enhances the binding of TPA in vitro to the cross-linked fibrin and fibrin elements within a matrix,[22] and at the same time weakens the fibrin cross-links.

Although low kilohertz frequencies better potentiate TPA effects,[23] these systems are not available for clinical practice because of safety concerns and the inability to image vasculature with this frequency/wavelength range. Meanwhile, frequencies of 1–2.2 MHz can also enhance TPA-induced thrombus dissolution through various mechanisms, such as fluid streaming around clot surface, disaggregation of fibrin fibers, and creating more binding sites for TPA without heating or cavitation.[24,25] This frequency range is safely and routinely used for diagnostic ultrasound examinations.

DIAGNOSTIC APPLICATIONS OF ULTRASOUND IN ACUTE CEREBRAL ISCHEMIA

Portable diagnostic 2-MHz transcranial Doppler (TCD) equipment can be used in the emergency room to continuously monitor TPA infusion in acute ischemic stroke patients.[26] With prior training and experience in the interpretation of TCD, this test, particularly in combination with urgent carotid and vertebral duplex scanning, can yield a high degree of accuracy for bedside detection and localization of arterial occlusion and recanalization.[26,27] In addition, TCD can be complementary to other imaging modalities, such as computed tomographic (CT) angiography by showing real-time flow findings (e.g., embolization, collateralization of flow with extracranial internal carotid artery disease, alternating flow signals indicative of steal phenomenon).[28] Finally, real-time flow findings during TCD-monitoring are predictive of the long-term functional outcome in ischemic stroke.[29,30]

Once abnormal residual flow signals are identified, an ultrasound beam can be steadily focused at the presumed intracranial thrombus location, and arterial recanalization can be monitored in real time.[26] In work at our institution, intravenous TPA infusion was continuously monitored with 2-MHz TCD[26] and early recanalization and dramatic recovery rates higher than expected were observed.[26] Two other studies conducted by separate groups in Germany[31] and France[32] also reported higher rates of recanalization than expected in patients with acute middle cerebral artery occlusion who were not eligible for TPA and received 2-MHz transcranial color-coded Doppler continuous monitoring. These provocative findings suggest a potential therapeutic effect of diagnostic transcranial ultrasound in the setting of acute cerebral ischemia and have led to the design of prospective randomized clinical trials.

THERAPEUTIC APPLICATIONS OF ULTRASOUND IN ACUTE CEREBRAL ISCHEMIA

Transcranial Doppler ultrasonography

The CLOTBUST trial (which stands for Combined Lysis Of Thrombus in Brain ischemia using transcranial Ultrasound and Systemic TPA) (FIG. 1) was a phase II clinical randomized multicenter international trial with centers in Houston (United States), Barcelona (Spain), and Edmonton and Calgary (Canada).[31] It had prespecified safety and signal of efficacy endpoints and a predetermined sample size of 63 patients per group.[33]

All enrolled patients had an acute ischemic stroke due to occlusion of the middle cerebral artery, and were treated with a standard 0.9 mg/kg dose of intravenous TPA therapy within 3 hours of symptom onset. They were randomized (1:1) to continuous TCD monitoring (Target) or placebo monitoring (Control). Emitted-power output was set at the maximal achievable level with selected insonation depths under the FDA-allowed threshold of 750 mW. Sample volumes, or gates of insonation, were set at 3–6 mm for power–motion Doppler units and 10–15 mm for all other single-channel 2-MHz TCD units.

The safety endpoint was symptomatic intracranial hemorrhage (sICH) causing worsening of the neurologic deficit by $\geq$4 points on the NIHSS. The primary combined activity endpoint was complete recanalization on TCD or dramatic clinical recovery by the total NIHSS score $\leq$3 points, or improvement by $>$10 NIHSS points within 2 hours after the TPA bolus. Clinical investigators were blinded to group assignment (active or sham monitoring) done by sonographers.

All projected patients (n = 126) received TPA and were randomized 1:1 to target or control (with median pretreatment NIHSS score of 16 points or 17 points, respectively). Age, occlusion location on TCD, and time to TPA bolus were similar between groups. Symptomatic

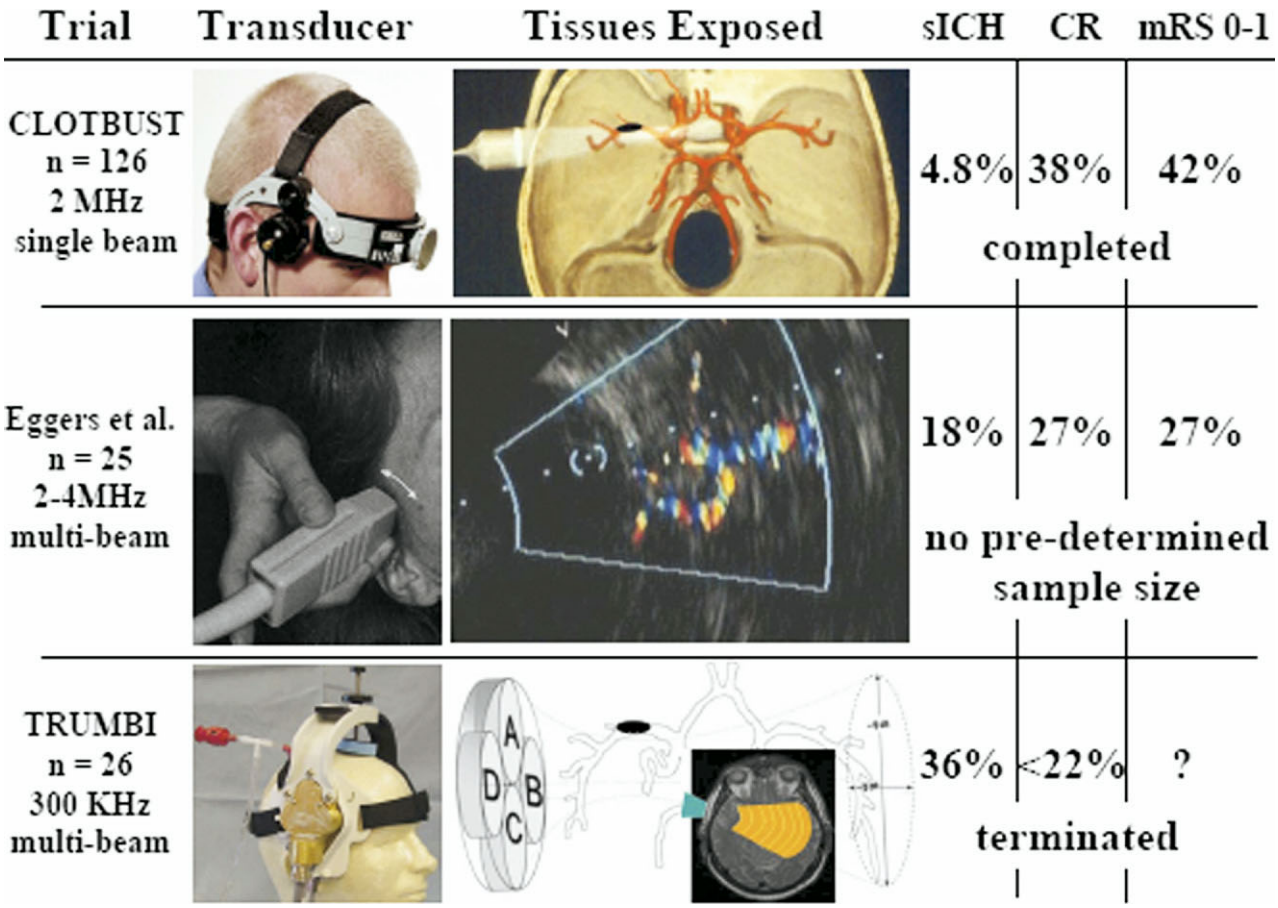

FIG. 1. Controlled clinical trials of ultrasound-enhanced systemic thrombolysis for acute ischemic stroke: Alexandrov et al. (CLOTBUST trial),[31] Eggers et al.,[32] and Daffertshofer et al. (TRUMBI trial).[39] The images under Transducer show the actual sources of ultrasound and their size relative to patient head; the images under Tissues Exposed show the beam paths as ultrasound propagates through the brain. *Abbreviations:* CR, complete recanalization at the end of monitoring period; kHz, kilohertz; mRS 0–1, modified Rankin scores at 3 months follow-up; n, total number of patients enrolled in both control and target groups; sICH, percent rates of symptomatic intracranial hemorrhages; ?, unknown (not reported in the original publication). Reproduced with permission from Alexandrov 2006.[51]

ICH occurred in 4.8% of Target and an equal 4.8% of Control patients. The primary endpoint was achieved by 31 Target patients (49%) and 19 Control patients (30%) ($P = 0.03$). At 3 months, 42% of Target and 29% of Control patients achieved favorable outcomes (mRS 0–1 points, $P = 0.20$). This trend indicates the feasibility of a pivotal phase III clinical trial that, at 274 patients per group, would be properly powered to detect this difference in outcomes at 3 months.[31]

The main limitation of the CLOTBUST trial was the extreme dependence on the skill of the TCD operator, which makes these results difficult to generalize. Vascular tests that require the use of such equipment are among the most difficult to perform, and it is unrealistic to expect that an average clinician can quickly develop skills for the rapid location of occlusions with this nonimaging, hand-held diagnostic method. Hence, only a small number of stroke centers had experienced sonographers on hand before TPA therapy was begun, and thus very few qualified for the CLOTBUST and related trials.

Transcranial color-coded duplex ultrasonography

Transcranial duplex technology was tested in a smaller randomized clinical trial.[32] Duplex transducers are different from those used in CLOTBUST, in that they generate multiple small beams at dual emitting frequencies, one for Doppler and one for grayscale imaging (FIG. 1). A major limitation of this technology is that there are no reliable head frames for transducer fixation, and most studies have to be performed hand-held. In addition, the mechanical index of these scanners is higher than TCD, and no dose escalation study was performed to determine how little ultrasound is needed to enhance thrombolysis without invoking safety concerns.

Eggers et al.[32] evaluated 25 patients (11 Target TPA + duplex monitoring, 14 Control TPA alone) and reported a trend in the Target group toward higher recanalization rates, more hemorrhagic transformations (18% sICH rate), and better neurologic outcomes at 3 months than for patients who received TPA alone. This study did not have a predetermined sample size, and the results may

have been affected by the small number of patients enrolled. More studies are needed to evaluate the potential of transcranial duplex technology to enhance thrombolysis.

This same group and others[33–35] reported provocative findings that patients who are not eligible for systemic TPA therapy may benefit from continuous monitoring with ultrasound alone because, hypothetically, ultrasound may help facilitate the endogenous thrombolytic process that leads to spontaneous recanalization in patients with acute stroke. It is unclear if only partial recanalization can be induced by ultrasound alone, and if this exposure would result in a significant difference at 3 months, to justify a large clinical trial.

Regardless, there are no clear data regarding the benefit of ultrasound monitoring without TPA. Thus, ultrasound-alone treatment should not be substituted for TPA treatment in patients otherwise eligible for thrombolytic therapy within 3 hours of symptom onset. Furthermore, different experimental strategies are being tested in an extended time window for acute stroke treatment and continuous exposure to ultrasound may find its application in combination with other strategies such as glycoprotein IIb-IIIa antagonists, direct thrombin inhibitors.

Intra-arterial ultrasound devices

Ultrasound transducers have been incorporated into catheters for intra-arterial delivery of a thrombolytic drug (NeuroWave catheter; EKOS Corporation, Bothell, WA). This intra-arterial device uses 1.7–2.1 MHz pulsed-wave ultrasound with the emitting power of 400 mW, specifications similar to extracranially applied TCD. The EKOS catheter is now being tested in phase II–III Interventional Management of Stroke (IMS) trials.[36]

Therapeutic low-frequency ultrasound

In experimental models, using lower frequencies (20 kHz to 1 MHz), TPA-mediated clot degradation was as much as 50% more efficient when ultrasound was added.[17–19] Consequently, it has been postulated that the use of therapeutic (i.e., nonimaging) ultrasound, in combination with intravenous thrombolytic therapy might be a feasible, safe, and potentially effective acute stroke treatment option.[37,38]

This hypothesis was tested in the TRUMBI trial (which stands for TRanscranial low-frequency Ultrasound-Mediated thrombolysis in Brain Ischemia).[39] At first, the investigators used a very low kilohertz system (<40 kHz), which produced intolerable tinnitus and was withdrawn from clinical testing (M. Daffertshofer, personal communication). This was replaced by a mid-kilohertz system operating at 300 kHz (FIG. 1). Low-frequency ultrasound (300 kHz, ± 1.5 kHz to avoid standing waves) with an intensity of 700 mW/cm^2 (temporal average spatial peak intensity) was applied simultaneously with intravenous administration of TPA and for 30 minutes after TPA infusion (total insonation time of 90 minutes).

The transducer had four elements arranged in a diamond pattern. The average temporal pressure was <1 atmosphere (<101 kPa), thereby avoiding cavitation. The mechanical index was <0.2. The low intensity levels, combined with the low frequency, resulted in a thermal index in soft tissue of <0.5 and a cranial thermal index of ~4. The higher cranial thermal index was addressed by means of both a cooling pad and a thermal sensor to detect excessive heating. To further reduce thermal effects, ultrasound was emitted in a pulsed fashion, with a 5% duty cycle and a pulse repetition frequency of 100 Hz (giving a cycle/pulse ratio of 225).

The trial was terminated after 26 patients were enrolled, with a 36% rate of symptomatic hemorrhage in the Target group and no signal of efficacy on early recanalization or clinical outcomes at 3 months. The investigators found a high rate of atypical hemorrhages in the TPA-plus-ultrasound group either in the subarachnoid or in the ventricular space or at remote parenchymal locations distant to the infarct core.

The underlying mechanism causing the high rate of hemorrhage was not clear. The high rates of subarachnoid hemorrhage particularly led to speculation of some mechanical action from the ultrasound disrupting small vessels in the subarachnoid space. Further research should determine if standing pressure waves and endothelial disruption may cause these adverse effects. If confirmed in *in vivo* models, this will have implications on design of future kilohertz-based systems.

In conclusion, the TRUMBI trial demonstrated adverse bioeffects of mid-kilohertz ultrasound in terms of promoting bleeding both in areas affected and unaffected by ischemia.

Microsphere-potentiated, ultrasound-enhanced thrombolysis

Experimental data have suggested that ultrasound-enhanced thrombolysis can be further amplified by adding gaseous microspheres. Gaseous microspheres (safe ultrasound contrast agents) are micron-sized lipid shells that, when exposed to ultrasound, expand and produce stable cavitation with stronger reflected echoes.[40–42] This is used to generate ultrasound images with better resolution. At the same time, microspheres agitate fluid where they are released by ultrasound, and this is useful in drug delivery and mechanical grinding of a thrombus. Microspheres have their own ability to lyse thrombi without a lytic drug.[40]

TABLE 1. *Controlled clinical trials of microsphere-potentiated ultrasound-enhanced systemic thrombolysis for acute ischemic stroke using transcranial doppler and transcranial color-coded ultrasonography*

Trial	Freq.	ECA	Design	Rand.	Active Treatment Group		
					ReC	AsxICH	sICH
Transcranial Doppler ultrasonography							
Molina et al.[43]	2 MHz	galactose-based	US/MS/TPA (n = 38) vs. US/TPA (n = 37) vs. TPA (n = 36)	No	71%	23%	3%
Alexandrov et al.[44]	2 MHz	perfultren lipid	US/MS/TPA (n = 12) vs. US/TPA (n = 3)	Yes	42%	25%	0%
Transcranial color-coded ultrasonography							
Larrue et al.[46]	2 MHz†	galactose-based	US/MS/TPA (n = 9) vs. TPA (n = 11)	Yes	48%	78%	0%
Perren et al.[45]	2 MHz†	phospholipid-encapsulated SF_6	US/MS/TPA (n = 11) vs. TPA (n = 15)	No	64%	NA	9%

Abbreviations: AsxICH, asymptomatic intracranial hemorrhage; mRS, modified Rankin Scale; MS, microspheres; NA, not available; Rand., randomization; ReC, recanalization at the end of TCD monitoring; sICH, symptomatic intracranial hemorrhage; TPA, tissue plasminogen activator; US, continuous ultrasound monitoring; ECA, echocontrast agent.
*Active treatment group: microsphere-potentiated ultrasound-enhanced systemic thrombolysis. †Patients received monitoring with a pulsed-wave, 2-MHz, phased-array Doppler and intermittent exposure to dual-frequency duplex.

Several published studies have used different commercially available microspheres (TABLE 1).[43–46] Molina et al.[43] pioneered this approach in stroke patients and reported the largest study to date that compared the CLOTBUST Target arm to the CLOTBUST Target insonation protocol (2 MHz continuous TCD-monitoring) combined with Levovist air microspheres (Schering, Berlin, Germany). Investigators demonstrated that at 2 hours after TPA bolus the TPA + TCD + Levovist group achieved a 55% sustained recanalization rate, compared with 38% in the TPA + TCD group of the CLOTBUST trial.

The safety and feasibility of infusion of a new and more stable octofluoropropane (C_3F_8) perfultren lipid microspheres in patients treated with ultrasound-enhanced thrombolysis has been reported in a small phase IIA randomized clinical trial.[43] Notably, in 75% of patients the microspheres permeated to areas with no pretreatment residual flow, and in 83% of patients the residual flow velocity improved at a median of 30 minutes from start of microsphere infusion (range 30 seconds–120 minutes) by a median of 17 cm/s, or 118% above pretreatment values (FIG. 2). There was no sICH both in the target (TPA + 2-MHz continuous TCD-monitoring + microspheres) and control group (TPA + 2-MHz continuous TCD-monitoring). Moreover, after evaluation of TCD tracings as part of a secondary analysis, microspheres were moving at velocities higher than surrounding residual red blood cell flow in patients with middle cerebral artery occlusions (39.8 ± 11.3 *versus* 28.8 ± 13.8 cm/s, $P < 0.001$).[47]

Larrue et al.[46] recently randomized patients with acute (<3 hours) middle cerebral artery main stem occlusion as demonstrated by CT or magnetic resonance (MR) angiography to either transcranial duplex ultrasound continuous monitoring combined with intravenous galactose-based microspheres and TPA (combined treatment group), or TPA alone (control group). This trial was prematurely discontinued for safety reasons, because a high rate of asymptomatic intracerebral hemorrhage was demonstrated on gradient-echo MRI in the combined treatment group (78%).

None of the intracerebral hemorrhages was symptomatic, however, and the fact that asymptomatic hemorrhagic transformation in the setting of acute cerebral ischemia has not been associated with poor outcome both in the NINDS[48] and the ECASS trial[49] should be taken into account when interpreting the results of the former study. It is also unclear why a data safety monitoring board was not appointed for this study and why a dose de-escalation decision was not made (i.e., reduce time of exposure to ultrasound or reduce the dose of microspheres).

In its current design, the study does not clearly indicate whether excessive hemorrhagic transformation rate was attributed to duplex ultrasound or microspheres since control subjects received TPA alone, without ultrasound. Finally, although the authors reported using continuous 2-MHz ultrasound monitoring, the term *2-MHz ultrasound* is misleading, in that dual-frequency duplex equipment was used and, every 10 minutes during treatment, color flow, and likely B-mode, were activated to reposition the sample volume. B-mode identification of the sylvian fissure and

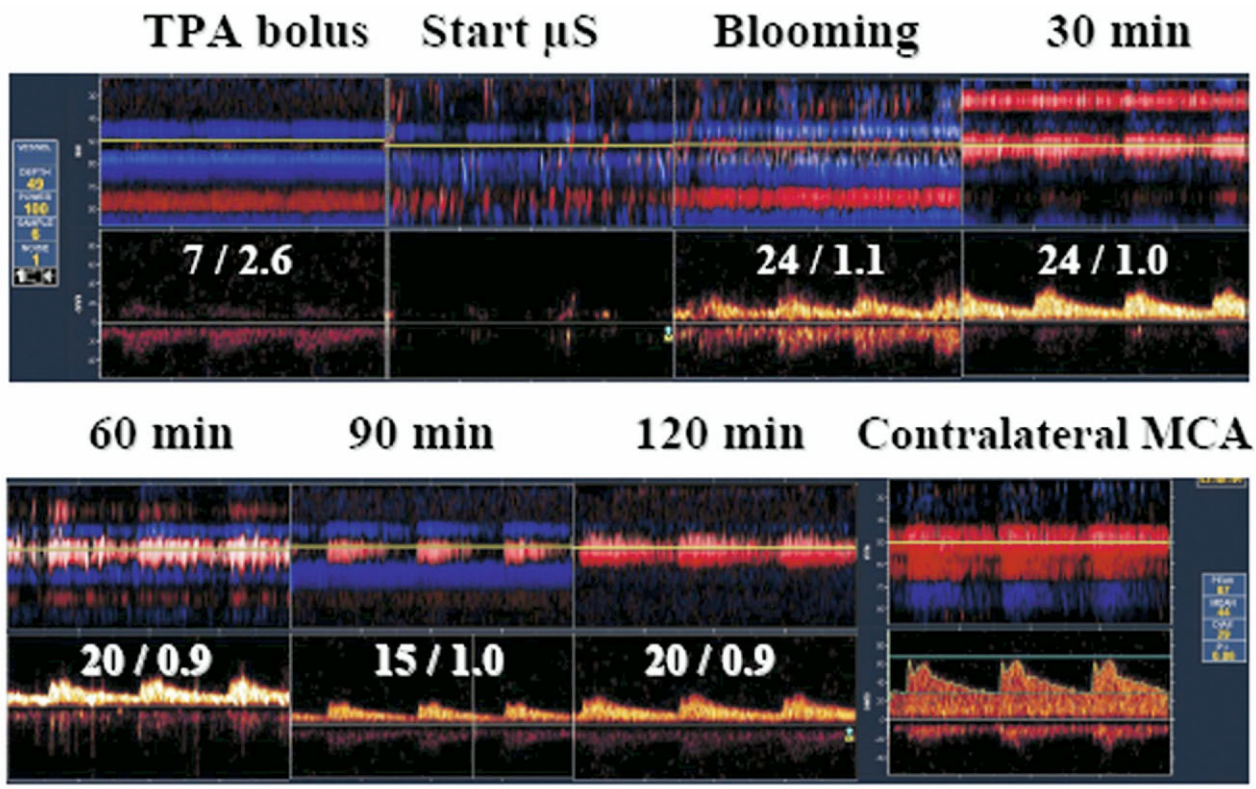

FIG. 2. Power–motion Doppler flow tracks showing individual and multiple perflutren lipid microsphere permeation to areas with no detectable residual flow pretreatment (white circles) and residual blood flow improvement following the administration of microspheres. *Abbreviations:* MCA, middle cerebral artery; TPA, tissue plasminogen activator; μS, microsphere infusion.

other structures introduced higher frequencies during treatment. Thus, this 2-MHz ultrasound should not be equated with other 2-MHz technologies, because by using a multitransducer phased array Doppler of essentially echocardiographic ATL (Advanced Technology Laboratories) transducer, the authors used multibeam technology that is different from TCD and that may not be completely free of other frequencies.

Finally, Perren et al.[45] studied the safety and feasibility of TCCD ultrasound monitoring combined with a second-generation, phospholipid-encapsulated sulfur hexafluoride (SF_6) microsphere (SonoVue; Bracco Diagnostics, Princeton, NJ) and intravenous systemic thrombolysis in patients with acute middle cerebral artery occlusion. All patients were monitored with continuous hand-held 2-MHz TCCD (pulsed-wave mode power output: 189 mW/cm^2; sample volume 10 mm; Acuson Sequoia; Siemens, Malvern, PA) over 60 minutes. Patients who received microsphere-potentiated ultrasound-enhanced thrombolysis seemed to fare better in terms of improvement in NIHSS score and to show sustained improvement in residual blood flow, compared with patients treated with TPA alone.

A phase I–II randomized placebo-controlled, open-label international multicenter study (TUCSON trial) of new and more stable perfultren lipid microspheres is underway (MRX 801; Imarx Therapeutics, Tucson, AZ; http://www.imarx.com/ImaRx/clinical_trials5_0). A total of 72 patients with acute intracranial arterial occlusion as demonstrated by CT or MR angiography will be randomized to microsphere-potentiated ultrasound-enhanced thrombolysis (4 groups with increasing doses of perfultren lipid microspheres) *versus* TPA alone.

FUTURE DIRECTIONS

Microspheres offer a mechanical way to amplify stroke therapies, and can be developed as novel neurotherapeutics to augment brain perfusion and drug and nutrient delivery within the existing ischemic penumbra. Microspheres offer also the potential of extending the time window for therapeutic acute stroke strategies—a high-priority research prerogative.[50] One obstacle to developing ultrasound and microsphere-assisted stroke therapies is the need for an experienced sonographer to locate the intracranial thrombus and expose its surface to residual flow, in order to lodge more TPA and to agitate stagnant flow. Personnel with these skills are lacking in most acute-care centers. Future studies should focus on the development of an operator-independent ultrasound device

that can be used by existing medical personnel regardless of their experience in diagnostic ultrasound.

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