Ultrasound- and Microspheres-Enhanced Thrombolysis for Stroke Treatment: State of the Art

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Abstract Intravenous administration of recombinant tissue-plasminogen activator (rt-PA) remains the fastest and widely feasible strategy to initiate treatment in acute ischemic stroke. Because it works by inducing recanalization of an occluded vessel, augmentation of this process is desirable and diagnostic transcranial Doppler (TCD) ultrasound can accomplish this safely. A 2-MHz pulsed-wave monitoring can at least double the chance of early complete arterial recanalization at no increase in the risk of symptomatic intracerebral hemorrhage. Gaseous microspheres, initially developed as ultrasound contrast agents, can further increase the effectiveness of rt-PA. A recent microsphere dose-escalation study called TUCSON showed sustained complete recanalization rates of 67% in patients receiving TCD monitoring with a 1.4-mL perflutren-lipid microsphere dose compared with controls receiving rt-PA alone with no increase in hemorrhage rate. In this article, we review the current and emerging applications of ultrasound and microspheres in stroke management including augmentation of systemic thrombolysis and implications for future reperfusion strategies.

Clinical Trial Acronyms

CLOTBUST	Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic TPA
IMS	Interventional Management of Stroke
TRUMBI	Transcranial Low-Frequency Ultrasound-Mediated Thrombolysis in Brain Ischemia
TUCSON	Transcranial Ultrasound in Clinical Sonothrombolysis

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Introduction

Systemic recombinant tissue-plasminogen activator (rt-PA) within a 3-hour window is the only established therapy for acute ischemic stroke in the United States, Canada, and Europe [1]. The recently published phase 3 trial showed that intravenous rt-PA between 3 to 4.5 h after symptom onset improves clinical outcomes in acute ischemic stroke at a small risk of symptomatic intracranial hemorrhage (sICH) [2••]. This is the second positive trial of intravenous rt-PA in acute ischemic stroke, and time window expansion for rt-PA is now endorsed by the US and European guidelines [3, 4]. The benefit of intravenous rt-PA remains time-dependent and we still should aim to treat patients as fast as possible.

Conversely, intra-arterial reperfusion procedures have the potential to deliver the thrombolytic agent inside the clot at a reduced dose, or mechanically remove the thrombus [5–8]. However, this approach can only be performed at relatively few hospitals capable of providing acute endovascular therapy; also, even if this rescue is approved in the future, it is unrealistic to expect that all patients with middle cerebral artery (MCA) occlusions will reach comprehensive stroke centers in time or that their risk factor profile would always make catheter intervention feasible [5–8].

Therefore, the intravenous route also remains the fastest and widely feasible way to initiate treatment particularly at a primary stroke center level, and there is a further need to amplify the systemic part of reperfusion therapy. Recent

expansion of therapeutic applications of ultrasound occurred in the field of sonothrombolysis and microspheres for assessing and treating stroke patients. We review the current and emerging applications of ultrasound and microspheres in stroke management.

Enhancing Recanalization

Thrombolytic stroke therapy is based on the "recanalization hypothesis" (ie, reopening of occluded vessels improves clinical outcome through regional reperfusion and salvage of threatened tissues). Because intravenous rt-PA by itself works through induction of mostly partial recanalization [9–11], early augmentation of fibrinolysis is desirable. The occurrence of early vessel recanalization and subsequent reperfusion are linked powerfully to final clinical outcome in acute ischemic stroke [12].

However, a proximal arterial recanalization does not necessarily lead to brain tissue reperfusion [13•]. Lack of reperfusion after early recanalization may be caused by downstream embolization, blockage of microcirculation due to no-reflow phenomenon, or rapid recruitment of infarcted tissue even before recanalization resulting in non-nutritional reperfusion. It has been hypothesized that sudden tissue reperfusion may be deleterious, leading to brain–blood barrier disruption and hemorrhagic transformation or massive brain edema from the so-called "reperfusion injury." Nonetheless, sudden reperfusion in rt-PA–treated stroke patients can safely lead to early neurologic recovery likely due to faster occurrence from symptom onset [10].

Fibrin strands are cross-linked and held by activated platelets to form a fisherman's net-like structure that captures red blood cells. This net is formed by nature to stop bleeding. In case of ischemic stroke, thromboembolic material lodges in the wrong place at the wrong time. Delivery of rt-PA to and through the thrombus appears dependent on the miniscule residual blood flow around the thrombus and plasma flow through the thrombus.

The Role of Ultrasound

Diagnostic Applications

Ultrasound expands the ability of clinicians to detect, localize, and quantify disease and to evaluate hemodynamic responses as a real-time tool for a neurovascular examination at bedside. With extensive training in transcranial Doppler (TCD), this test, particularly in combination with urgent carotid/vertebral duplex scanning, can yield a high degree of accuracy for bedside detection and localization of arterial occlusion and real-time monitoring of recanalization [9]. In addition, TCD can be complementary to other "static" imaging modalities such as CT angiography by showing real-time flow findings (eg, embolization, collaterals, alternating flow signals) [14]. Real-time changes in cerebral hemodynamics can predict neurologic deterioration [15].

Recently, our group described an intracranial hemodynamic steal (reversed Robin Hood syndrome [rRHS]) [16•] particularly common in patients with persistent proximal arterial occlusions and daytime sleepiness. These patients may develop recurrent neurologic symptoms in response to vasodilatory stimuli such as hypercapnia or reductions in blood pressure/cardiac output when normal vessels dilate first, thereby reducing pressure gradient through collaterals and worsening brain perfusion. This mechanism could link hypoventilation, sleep apnea, persisting occlusion, and neurologic deterioration after stroke. This steal phenomenon can be detected and characterized by TCD in real time. If the steal is confirmed as the cause of neurologic worsening, rRHS may identify a target group for testing blood pressure augmentation and noninvasive ventilatory correction in stroke patients [16•].

TCD also depicts abnormal residual flow waveforms at the intracranial thrombus location. These waveforms are used to identify the monitoring site and grade recanalization process in real time, finding predictive outcomes after acute cerebral ischemia [9].

Therapeutic Applications

Besides detection of occlusion, recanalization, and reocclusion [9], ultrasound as a pressure wave can travel through tissues and deliver its mechanical momentum to stagnant flow areas and clot interfaces. Mechanical agitation can expose shallow layers of thrombi to circulating rt-PA and facilitate streaming of plasma through thrombus, thus bringing more rt-PA to binding sites. This can be achieved even with a low-power ultrasound of the diagnostic frequency range. Numerous experiments confirmed the ability of ultrasound to enhance fibrinolysis through disaggregation and thinning of uncross-linked fibrin strands, thus promoting plasma flow and lytic drug delivery to the clot [17, 18].

Although first therapeutic applications for ultrasound were thought of as early as the 1920s, the potential for ultrasound to dissolve intra-arterial thrombi started to capture scientists' attention in the 1970s [18, 19]. The ability of ultrasonic waves to enhance thrombolysis was demonstrated by pioneering groups [17–20] and confirmed by several investigators in experimental models [21, 22]. Among several possible mechanisms of action, ultrasound can promote the motion of fluid around the thrombus, an effect called streaming [23]. Exposure to ultrasound increases uptake of rt-PA into clots with deeper penetration. Ultrasound energy promotes the mixing of rt-PA and

increases its concentration within the thrombus. Thus, hypothetically, ultrasound can promote rt-PA delivery despite stagnant flow near the intracranial occlusion [24]. In addition, ultrasound waves directly affect binding of rt-PA to fibrin mesh [25], simultaneously weakening fibrin cross-links.

Various ultrasound energies (0.2–2.0 W/cm^2) and frequencies (20 kHz–2 MHz) have been tested. Kilohertz frequencies likely induce more mechanical stretching, whereas megahertz ultrasound promotes fibrinolysis more through enzymatic mechanisms [21, 26]. Although low kilohertz frequencies better potentiate rt-PA effects, these systems are not available for clinical practice because of safety concerns [22] and inability to image vasculature with this frequency range. Combination (intermittent kHz–MHz) devices are being tested in experiments and early safety clinical studies (Furuhata, Personal communication). Meanwhile, 1- to 2.2-MHz frequencies can also enhance thrombolysis [27], and this frequency range is safely and routinely used for diagnostic ultrasound examinations and for monitoring stroke patients.

Clinical Studies of Sonothrombolysis

Early recanalization and dramatic recovery rates were first observed in stroke patients when our group started monitoring rt-PA infusion with 2-MHz TCD [9]. In the past 10 years, early partial recanalization coupled with clinical recovery were also observed by others [26, 28]; this included patients ineligible for rt-PA who received transcranial duplex monitoring, suggesting a potential benefit from exposure to ultrasound alone. It remains unclear whether only partial recanalization can be induced by ultrasound alone and if ultrasound exposure could affect clinical outcome. In any case, at present no clear data exist regarding the benefit of ultrasound monitoring without rt-PA. Systemic thrombolysis with rt-PA remains the mainstay therapy within the first 4.5 h and should not be substituted with ultrasound monitoring outside a rigorous clinical trial.

Eggers et al. [28] randomized 25 patients (11 rt-PA + duplex monitoring, 14 controls with rt-PA alone) and reported a trend toward higher recanalization rates, more hemorrhagic transformations (18% sICH), and better neurologic outcomes at 3 months with ultrasound. However, duplex technology has some disadvantages: 1) multiple beams at dual-emitting frequencies may create standing waves; 2) no reliable head frames for transducer fixation with most studies using handheld probes; and 3) higher mechanical index than TCD.

The CLOTBUST trial was a phase 2, clinical, randomized, multicenter trial conducted in Houston, Texas; Barcelona, Spain; Edmonton, Canada; and Calgary, Canada [11]. This trial was the first properly powered clinical trial that confirmed the existence of ultrasound-enhanced thrombolysis in humans and demonstrated a positive biological effect of diagnostic ultrasound. A total of 83% of patients achieved any recanalization (46% complete, 27% partial) with rt-PA plus TCD versus 50% (17% complete, 33% partial) with rt-PA alone within 2 h of treatment ($P<0.001$). Sustained complete recanalization at 2 h was 38% versus 13%, respectively ($P=0.03$). The sICH rate was 3.8% in both groups (not significant).

Another clinical trial TRUMBI [29] was stopped prematurely because of excessive sICH rates with sonication at 300 kHz. Also, MRI detected any hemorrhages in 93% of the target and 42% control patients. The reason for hemorrhages in the TRUMBI trial remains unclear. An experiment in rats was unable to demonstrate harmful effects of ultrasound using the TRUMBI parameters [30]. One study suggested that hemorrhages in TRUMBI were related to abnormal permeability of the human blood–brain barrier that was induced by wide-field low-frequency insonation and standing waves [31].

A meta-analysis of six randomized and three 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 [32•].

Currently, experienced sonographers have to perform urgent ultrasound tests to localize occlusions using spectral Doppler residual flow waveforms (Thrombolysis in Brain Ischemia system) [33] or B-mode/color flow duplex imaging [26]. For sonothrombolysis to be applied in any emergency room ultrasound, targeting should be operator-independent [34], and imaging technologies can be further improved [35–38]. Recent advances include contrast microspheres tagged to recognize and to adhere to the thrombus [35], crossed beams under magnetic resonance image guidance [39], and direct imbedding into the clot [40] by intraclot delivery via catheters such as the EKOS technology (EKOS Corp., Bothell, WA) [40]. The EKOS MicroLysUS infusion catheter is a 2.5 F standard microinfusion catheter with a 2-mm, 1.7- to 2.1-MHz pulsed-wave ring transducer (average power, 0.21–0.45 W; spatial peak temporal average intensity, 400 mW/cm^2) at its distal tip. This transducer generates a 360° circumferential pulse wave in conjunction with intra-arterial thrombolytic infusion [40].

The EKOS microcatheter is feasible for the treatment of acute ischemic stroke [41]. In a pilot study, the average time to recanalization was 46 min, with 8 of 14 patients achieving Thrombolysis in Myocardial Ischemia flow grades of 2 or 3 within the first hour. Overall clinical outcomes as measured by modified Rankin Scale grades of 2 were similar to historical controls. Mortality (36%) and sICH (14%) rates compared reasonably with those reported in previous trials of

intra-arterial thrombolysis. The EKOS catheter is now being tested in a phase 3 IMS trial [42] that has a drug-device combination (intravenous rt-PA–intra-arterial reperfusion) arm developed after the IMS II study [43].

Gaseous Microspheres

Since the first description of enhanced reflections of ultrasound in the aortic root after injection of small air bubbles by Gramiak and Shah [44], contrast ultrasound agents underwent rapid development. Air bubbles introduced without a stabilizing shell were very short lived, and therefore methods to stabilize the gas-liquid interface were developed. Simultaneously, low diffusivity gases were introduced to further increase the microsphere circulation time. Clinical ultrasound systems now incorporate contrast-specific imaging modes designed to take advantage of unique nonlinear properties of these small gas nuclei. Microspheres have now been in clinical use as contrast agents for ultrasound imaging for almost 30 years. Today, these stabilized gas bubbles are used in applications spanning cardiology, radiology, and cerebrovascular disease [45].

The development of harmonic imaging together with advances in three-dimensional visualization techniques have further enabled mapping of the microcirculation using microspheres particularly in the brain, offering real-time advantages for assessing stroke patients [46, 47]. Microspheres enable measurement of parameters such as relative vascular volume, flow velocity, and perfusion rate. The ability to attach molecules to microspheres that are targeted to specific vascular receptor sites has opened up further opportunities for molecular imaging; conditions currently under investigation include inflammation, angiogenesis, and atherosclerosis [48, 49].

Perhaps one of the most exciting therapeutic applications of microspheres is sonothrombolysis, in which they markedly increase rt-PA–induced clot lysis in vitro and in vivo [50, 51]. The latest generation of gaseous microspheres are micron-sized lipid shells that when exposed to ultrasound expand, oscillate, or collapse producing stable cavitation and stronger reflected echoes [46, 51]. This is used to generate ultrasound images with better resolution. At the same time, microspheres agitate fluid in which they are activated by ultrasound, and this is useful in drug delivery and mechanical "grinding" of a thrombus. In fact, microspheres have their own ability to lyse thrombi without a lytic drug [52].

In vitro experiments with the temporal bone/arterial flow model showed that even a low-power and attenuated-by-bone TCD is able to destroy some of the microspheres, and reflections even from a single oscillating microsphere are possible to trace with frequencies similar to conventional TCD equipment [53]. When microspheres oscillate or burst, they expand like microscopic balloons [54], move in three dimensions, and transmit mechanical energy momentum from passing ultrasound wave to surrounding fluid and structures. If an ultrasound beam intercepts microspheres at the clot-residual flow interface, this could facilitate fibrinolysis with or even without rt-PA (Figs. 1 and 2) [51].

Another exciting role for ultrasound-microsphere combination was proposed for modulation of the excitotoxic cascade after ischemic stroke using 2-MHz ultrasound and second-generation, sulfur hexafluoride microspheres in rats [55]. In the treated group, infarctions were smaller, lacking apoptotic cell death outside the infarction area with no hemorrhages, suggesting a neuroprotective effect of ultrasound and microspheres [55]. The postulated "neuroprotective mechanism" may depend on improvement of blood flow to penumbra via collaterals and changes in the microenvironment of damaged tissue, such as decreased cell damaging factors (eg, glutamate or enhanced enzyme activity of endothelial nitric oxide). Interestingly, before this study our group showed that a low-power 2-MHz TCD ultrasound can reduce infarction in rat brain after permanent MCA occlusion even without microspheres [56]. Therefore, the additive role of microspheres to ultrasound and ultrasound-alone–induced vasodilation through nitric oxide mechanism release need to be evaluated.

Molina et al. [50] pioneered coupling commercially available microspheres with TCD for treating acute stroke patients. His group compared the CLOTBUST target arm to a 2-MHz continuous TCD monitoring combined with Levovist air microspheres (Schering AG, Munich, Germany) [50]. Investigators demonstrated that at 2 h after rt-PA bolus, the rt-PA plus TCD plus Levovist group achieved a 55% sustained recanalization rate compared with 38% in the rt-PA plus TCD group of the CLOTBUST trial.

The safety and feasibility of infusion of new and more stable C_3F_8 (perflutren-lipid) microspheres in patients treated with ultrasound-enhanced thrombolysis has recently been reported in a small phase 2A randomized clinical trial [50]. Since then, several studies have been reported with different types of commercially available microspheres confirming higher recanalization rates with the addition of microspheres [50, 57–60].

In a pilot clinical trial of perflutren-lipid microspheres with concurrent controls, microspheres permeated to areas with no pretreatment residual flow in 75% of patients, and in 83%, residual flow velocity improved at a median of 30 min from the start of microsphere infusion (range, 30 s to 120 min) by a median of 17 cm/s, or 118% above pretreatment values [58]. No sICH was found in the target (rt-PA + 2-MHz TCD monitoring + microspheres) and in the control groups (rt-PA + 2-MHz TCD monitoring). Moreover, microspheres were moving at velocities higher
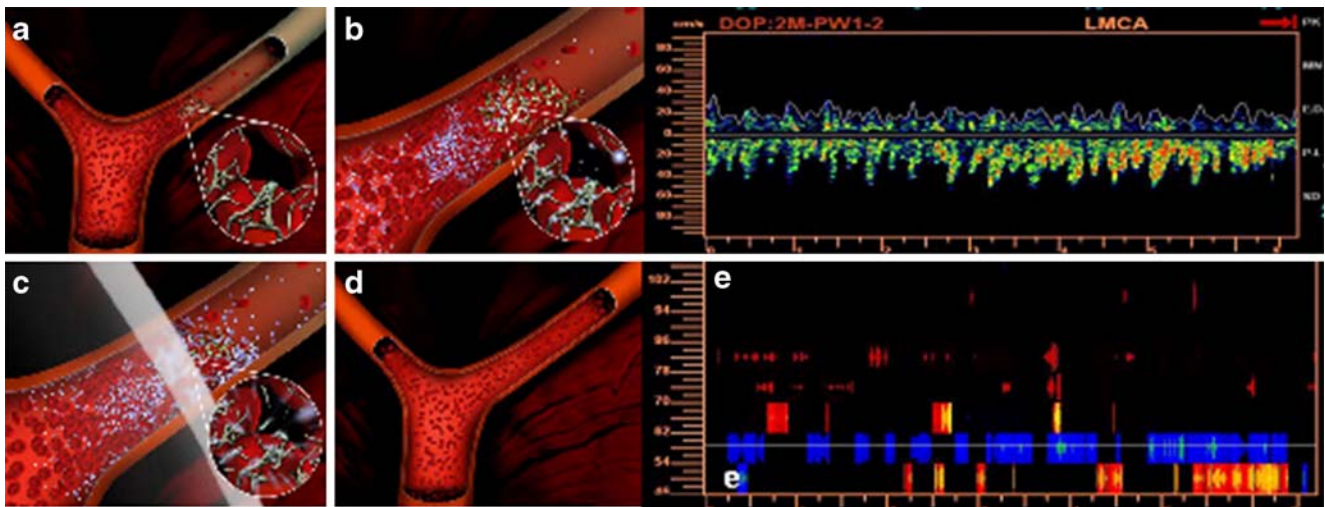
Fig. 1 a Intracranial occlusion. b Microspheres reaching the site of intracranial occlusion and permeating around or through the thrombus. c Ultrasound energy momentum destroying microspheres. d Complete arterial recanalization. e Spectral Doppler and motion mode flow while microspheres are reaching the site of intracranial occlusion and permeating around or through the thrombus. LMCA—left middle cerebral artery

than surrounding residual red blood cell flow in patients with MCA occlusions (39.8±11.3 vs 28.8±13.8 cm/s, $P<0.001$) [58]. The first dose of these microspheres was safely coadministered during rt-PA infusion via a separate arm causing no sICH. As a signal of efficacy, perflutren-lipid microspheres, TCD, and rt-PA completely lysed 50% of proximal MCA occlusions, which favorably compares with concurrent and historic controls receiving rt-PA alone [58].

Most recently, a multicenter microsphere dose-escalation study called TUCSON was completed [61]. Stroke patients with pretreatment proximal intracranial occlusions on TCD were randomized (2:1 ratio) to rt-PA with perflutren-lipid microsphere (MRX-801) infusion over 90 min (cohort 1, 1.4 mL; cohort 2, 2.8 mL) and continuous TCD insonation,

whereas controls received rt-PA and brief TCD assessments. The primary safety end point was sICH within 36 h after tissue plasminogen activator. Among 35 patients (cohort 1=12, cohort 2=11, controls=12), no sICH occurred in cohort 1 and controls, whereas three (27%, with two fatal) sICHs occurred in cohort 2 ($P=0.028$). Sustained complete recanalization rates at the end of TCD monitoring were 67% for cohort 1, 46% for cohort 2, and 33% for controls ($P=0.255$). The median time-to-any-recanalization tended to be shorter in cohort 1 (30 min/interquartile range [IQR]=6) and cohort 2 (30 min/IQR=69) compared with controls (60 min/IQR=5; $P=0.054$). Although patients with sICH had similar screening and pretreatment systolic blood pressure (SBP) levels compared with the rest, higher SBP levels were documented in sICH(+) patients at 30, 60, 90 min, and 24 to 36 h after rt-PA
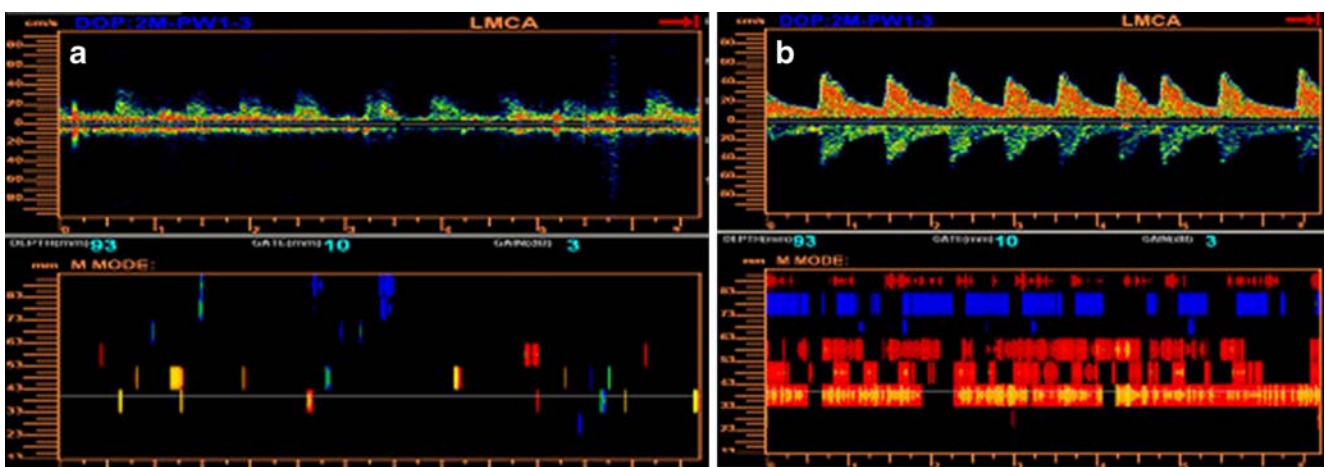

Fig. 2 a Spectral Doppler and motion mode flow before treatment with recombinant tissue-plasminogen activator plus ultrasound plus microspheres. b Subsequent recanalization with relative velocity increase due to microspheres. LMCA—left middle cerebral artery

bolus. Although the study was stopped halfway by the sponsor for administrative reasons, this trial showed that perflutren-lipid microspheres can be safely administered with rt-PA at the first dose tier, confirming previous pilot study results [58].

The rates of recanalization and clinical recovery tended to be higher in both microsphere dose tiers compared with controls. However, it should be noted that sustained complete recanalization rates at the end of TCD monitoring were higher in cohorts 1 and 2 without reaching statistical significance ($P=0.255$). Similarly, the differences between groups in terms of functional outcomes were not significant ($P=0.167$). In agreement with previous studies, the addition of microspheres to rt-PA and ultrasound monitoring should reach at least 50% complete recanalization rate for proximal intracranial occlusions [45, 57, 58, 62••].

Finally, Ribo et al [61]. studied the safety and efficacy of local microsphere administration during intra-arterial thrombolysis and continuous TCD monitoring for MCA recanalization. After no recanalization was achieved with intravenous rt-PA, nine patients underwent intra-arterial rescue procedures with the addition of microspheres, suggesting that combination of ultrasound and intra-arterial microspheres and rt-PA may be a strategy to enhance the thrombolytic effect and increase recanalization rates [61].

Conclusions

Enhancing the only approved systemic therapy for stroke is desirable, and microspheres together with ultrasound offer a promising way to reach this target. A combination of drugs and devices may also represent a more effective treatment initiation strategy with the goal of developing an intravenous–intra-arterial approach to recanalize thrombi most resistant to fibrinolysis alone.

To improve ultrasound and microsphere-assisted stroke therapies, a need exists to replace an experienced sonographer in the emergency situations with an operator-independent device that would reliably deliver ultrasound and activate microspheres at the thrombus-residual flow interface in often restless stroke patients. This would enable access to sonothrombolysis $24\times7\times365$ at primary and comprehensive acute care centers. With advances in microsphere dose finding and ultrasound technology, a phase 3 trial of sonothrombolysis may soon be a reality.

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