SONOTROMBOLYSIS: IS THE STORY (T)OLD OR JUST THE BEGINNING

SONOTROMBOLIZA: DA LI JE OVA PRIČA ISPRIČANA ILI UPRAVO POČINJE

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Summary

Introduction. Intravenous administration of recombinant tissue plasminogen activator, fastest and widely feasible treatment in acute ischemic stroke induces arterial recanalization, a prerequisite for neurological recovery. The Therapeutic Role of Ultrasound and Potential Mechanism of Sonothrombolysis. Augmentation of recanalization can be achieved safely in combination with diagnostic transcranial Doppler by delivering mechanical pressure waves to the thrombus and exposing more thrombus surface to circulating drug. The addition of microspheres can further improve thrombolytic effect. Clinical Trials. International multicenter CLOTBUST trial showed that acute ischemic stroke patients treated with sonothrombolysis had higher rate of arterial recanalization and dramatic clinical recovery without increasing risk of symptomatic intracranial hemorrhage. A microsphere dose-escalation study called TUCSON showed that rates of recanalization and clinical recovery tended to be higher in target groups compared with controls. Meta-analysis of clinical trials on sonothrombolysis. Cochrane Stroke Group found that sonothrombolysis was likely to reduce death or dependency. A meta-analysis of sonothrombolysis showed that patients who received any form of sonothrombolysis had more than twofold higher likelihood of achieving complete arterial recanalization. Perspectives for sonothrombolysis - Operator-independent device for sonothrombolysis. The collaborative group of the CLOTBUST trial designed multi-transducer assembly to cover conventional windows used for transcranial Doppler examinations. Operator-independent device can be quickly mounted by medical personnel with no prior experience in ultrasound. Sonothrombolysis for acute ischemic stroke is now tested in a pivotal efficacy multi-national trial called CLOTBUSTER. Conclusion. Ultrasound is a promising tool to enhance systemic thrombolysis.

Key words: Mechanical Thrombolysis; Ultrasonic Therapy; Microbubbles; Stroke; Thrombolytic Therapy; Ultrasonography, Doppler, Transcranial; Tissue Plasminogen Activator

Sažetak


Ključne reči: Mehanička tromboliza; Ultrazvučna terapija; Mikromehurići; Moždani udar; Trombolitička terapija; Transkranijalna Dopler ultrasonografija; Aktivator tkivnog plazminogena
Introduction

Intravenous recombinant tissue plasminogen activator (IV rtPA) is still the only approved treatment for acute ischemic stroke, which could be administered within the first 4.5 hours after symptom onset [1]. Treatment can be initiated fast in any emergency room equipped with a non-contrast computed tomography (CT) scanner [2]. In most medical centers in the developing countries, it is the only option for the treatment of acute ischemic stroke patients. On the other hand, transcranial Doppler (TCD) is a non-invasive, well-established and widely used ultrasound technique for fast evaluation of cerebral hemodynamics and real-time detection of arterial occlusions and recanalization during or after thrombolysis [3–7]. The high level of agreement between TCD and angiographic findings in acute ischemic stroke patients was shown in several studies [8, 9] and the patterns of intracranial arterial occlusion and recanalization on TCD are determined using the thrombolysis in brain ischemia (TIBI) grading system [8].

Various factors are associated with the outcome after acute ischemic stroke. Besides initial stroke severity, comorbidities, and patient age, the early recanalization of an acutely occluded blood vessel is associated with final infarct size, neurological improvement, and final clinical outcome [10–12]. Nevertheless, recanalization rates with IV rtPA alone are low, especially in patients who suffer major proximal occlusions [13, 14]. Several ways to improve the speed and completeness of recanalization have been studied, including the therapeutic use of ultrasound alone or in combination with IV rtPA [15]. Improved recanalization has been demonstrated with diagnostic TCD and transcranial color-coded sonography (TCCS) in combination with standard IV thrombolysis with rtPA in two randomized trials [13, 16]. Sonothrombolysis was introduced for treatment of acute intracranial occlusions at the turn of this century [17, 18].

The Therapeutic Role of Ultrasound and Potential Mechanism of Sonothrombolysis

The idea for the use of ultrasound as a thrombolytic agent is not new [14]. The ability of a mechanical pressure wave, i.e. ultrasound, to enhance thrombolysis was documented in 1976 [19] and since then has been confirmed by many experimental models [20]. However, despite numerous studies documenting a thrombolytic effect of ultrasound, the underlying mechanisms remain poorly understood [21]. The likely mechanism that emerged from these in vitro and in vivo experiments is the ability of ultrasound, through transmission of the mechanical energy momentum, to agitate flow and facilitate streaming of plasma around and through the thrombus, thus delivering more recombinant tissue plasminogen activator (rtPA) to the target clogging sites [22–26]. In stroke patients, ultrasound can promote rtPA delivery to the areas with diminished flow near occlusion and the pressure of ultrasound waves may increase the permeation of rtPA into the fibrin network [27].

Various ultrasound energies (0.2–2.0 W/cm²) and frequencies (20 kHz – 2 MHz) have been tested [28, 29]. Although low kilohertz frequencies potentiate rtPA effects better, these systems (a combination of rtPA with an experimental kHz delivery system) resulted in an excessive risk of intracerebral hemorrhage in stroke patients. The clinical trial TRUMBI (Transcranial Low-Frequency Ultrasound-Mediated Thrombolysis in Brain Ischemia) was stopped prematurely because of excessive symptomatic intracranial hemorrhage (sICH) rates with sonication at 300 kHz [30]. Kilohertz frequencies were thought to induce more mechanical stretching and minimize heating [22, 31]. On the other hand, 1–2.2 MHz frequencies can also enhance rtPA-induced thrombus dissolution, utilizing different mechanisms such as fluid streaming around the clot surface, disaggregation of fibrin fibers, and delivering more tPA to the binding sites without cavitation [22, 32]. Though some concerns remain regarding tissue heating, skull absorbs most of 2 MHz ultrasound energy and this frequency is safely used for diagnostic ultrasound examinations and monitoring [32, 33].

Furthermore, it has been shown that the addition of microbubbles composed of lipid, albumin, or galactose capsules to the combination of rtPA and ultrasound additionally enhances thrombolysis [34–36]. Microbubbles agitated by the same mechanism of cavitation and microstreaming can cause localized mechanical stress on the adjacent clot. Energy delivered to the microbubbles can
lead to their oscillation in size, disruption and production of "microjets" that are also effective in eroding a clot [21, 37, 38]. The mechanical thrombolytic effect has been confirmed in several experimental studies of only microbubbles and ultrasound, without thrombolytic drugs [39–42].

Clinical Trials

The study with low (kHz) frequency ultrasound (TRUMBI) showed a significant increase in SICH and the trial was stopped prematurely [30]. Since then, low frequency ultrasound has not been available for therapeutic purposes in clinical practice [14].

Early recanalization and dramatic recovery rates in acute ischemic stroke patients treated with rtPA were first observed during monitoring of cerebral blood flow with standard diagnostic 2-MHz TCD [17]. After identifying abnormal residual flow signals, an ultrasound beam was steadily focused on the presumed location of an intracranial thrombus, and arterial recanalization could be monitored in real time. Thus, the authors observed early recanalization and more dramatic recovery rates than expected, which suggested a potential therapeutic effect of TCD [17]. This pilot study led to the design of the phase II of CLOTBUST trial (Combined Lysis of Thrombus in Brain ischemia using transcranial Ultrasound and Systemic TPA) [13]. This randomized multi-center international clinical 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. It enrolled 126 patients with acute ischemic stroke due to occlusion of the MCA who were treated with IV rtPA therapy within 3 hours of symptom onset. All patients received IV rtPA and were randomized (1:1) to the target group receiving continuous TCD-monitoring or the control group with placebo monitoring during one hour IV rtPA infusion and one extra hour after rtPA was completed. A standard fast-track TCD examination was performed in the emergency department before rtPA bolus. TCD was used to identify the site of intracranial occlusion using TIBI grading system. Once the occlusion was diagnosed with handheld examination, the presumed clot location and residual flow around it were determined through the presence of one of the abnormal TIBI flow signals (minimal, blunted, or dampened waveforms). TCD monitoring or placebo monitoring was performed for two hours under direct visual control of the sonographer investigators. Recanalization was graded as complete, partial, or none according to TIBI criteria [8]. In both groups, follow-up measurements were performed 30, 60, 90, and 120 minutes after the rtPA bolus was given. The complete recanalization was seen in 38% of patients in the target group and in 13% of patients in the control group, p = 0.002. A total of 73% of patients achieved any recanalization with rtPA plus TCD versus 50% of patients with rtPA alone within 2 hours of treatment (P<0.001).

Neurological examinations with the National Institutes of Health Stroke Scale (NIHSS) and follow-up assessments were performed by neurologists unaware of monitoring group assignment. Symptomatic ICH occurred in 4.8% in both (target and control) groups. At 3 months, 42% of patients in the target group and 29% of patients in the control group achieved favorable outcomes (modified Rankin scale (mRS) grades of 0-1, p=0.2). The CLOTBUST trial was not powered to evaluate the efficacy of ultrasound enhanced thrombolysis in improving functional outcome, but was designed to establish the safety of this treatment and to provide preliminary data for the design of a larger clinical efficacy trial. The main limitation of the CLOTBUST trial was the operator-dependency and the need for a qualified sonographer to be present at bedside to find, aim and deliver ultrasound beam to the thrombus residual flow interface [2].

Transcranial color-coded duplex (TCCD) is another ultrasound method that provides images of both cerebral arterial locations with real-time cerebral blood flow and imaging of parenchymal structures inside the brain [14, 16, 43, 44]. TCCD has also been evaluated for ultrasound enhanced thrombolysis in smaller single-center randomized clinical trials and the authors observed a similar effect in the patients with acute cerebral artery occlusion [16, 45–47]. In the Lübeck study, Eggers et al. evaluated 37 patients who were randomly selected to receive TCCS-guided pulsed-wave (PW) mode ultrasound (US) for 1 hour [16]. There were 19 patients in the target group (IV rtPA + duplex monitoring) and 18 control patients (IV rtPA alone). The patients with proximal middle cerebral artery (MCA) occlusions who underwent simultaneously ultrasound insonation and rtPA standard treatment were included in the study. Similar to the findings of the CLOTBUST trial, a trend towards higher recanalization rates was reported in the target group. Treatment with combination of IV rtPA and TCCD resulted in significantly improved recanalization; partial or complete recanalization was detected in 57.9% of patients in the target group vs. 22.2% of patients in the control group (p = 0.045). Additionally, a better functional outcome after 3 months in target patients was shown. However, tendencies for increased symptomatic cerebral bleeding (three patients in the sonothrombolysis group vs. one patient in the control group) and increased hemorrhagic transformation of infarcts were also found in patients who underwent continuous insonation [16]. The same group of authors and others have reported provocative findings that patients who are not eligible for systemic rtPA therapy may potentially benefit from continuous monitoring with ultrasound alone since, hypothetically, ultrasound may help facilitate the endogenous thrombolytic process that...
leads to spontaneous recanalization in patients with acute stroke [46, 47]. In 15 patients ineligible for rtPA therapy, the recanalization after 1h occurred only in the sonothrombolysis group (62.5% in the sonothrombolysis group vs. 0% in the control group), followed by significant improvements in clinical course after 4 days and functional independence after 3 months (2 of 8 patients in the sonothrombolysis group compared with none of the 7 patients in the control group). There was no symptomatic ICH in the sonothrombolysis group [46]. However, duplex technology has some disadvantages: multiple beams at dual-emitting frequencies and higher frame rates may create standing waves, no reliable head frames for transducer fixation with most studies using handheld probes, and a higher mechanical index than TCD [48]. In the absence of a large controlled trial, there are no clear data regarding the benefit of ultrasound monitoring without rtPA. Thus, rtPA treatment should not be substituted for ultrasound alone in the patients otherwise eligible for thrombolytic therapy within 4.5 hours of symptom onset. Finally, additional studies are needed to evaluate and clarify the potential of transcranial duplex technology to enhance thrombolysis [49].

The data derived from experimental studies in the 90s have suggested that ultrasound-enhanced thrombolysis can be further amplified by microbubbles [34-36, 50]. Microbubbles, composed of lipid, albumin, or galactose shells, and ranging in size from 0.5 μm to 5 μm erode the surface of clots and lower the threshold for thrombolysis by different ultrasound mediated mechanisms (e.g., enhancement of microstreaming and cavitation) [51]. Microspheres, in combination with ultrasound can lyse thrombi even without a thrombolytic drug [52]. Numerous studies have shown significant amplification of thrombolysis with the addition of microspheres to the combination of thrombolytic drug and ultrasound [53]. Molina et al. pioneered the use of gaseous microspheres in combination with CLOTBUST monitoring methods in 38 patients compared to 73 patients treated with either 2 MHz TCD and IV rtPA or IV rtPA alone [54]. Complete recanalization rate 2 h after rtPA bolus was significantly higher in the rtPA + TCD + microspheres group (54.5%) compared with rtPA + TCD (40.8%) and rtPA alone (23.9%) (p = 0.038). Symptomatic ICH rates did not differ. Another study that evaluated the safety and feasibility of TCCD ultrasound monitoring combined with microspheres and IV rtPA in patients with acute middle cerebral artery occlusion reported improved recanalization flow [55]. Complete recanalization rate was 64% in comparison to 53% of patients treated with IV rtPA only. The safety and feasibility of infusion of newer generation of microspheres were tested in patients in a pilot trial and a phase IIa study [56-58]. A multicenter microsphere dose-escalation study called TUCSON (Transcranial Ultrasound in Clinical SOnothrombolysis) was aimed at determining the safety, tolerability, and activity of perflutren-lipid microspheres plus TCD sonication in sonothrombolysis [59]. The study showed a trend towards higher rates of early recanalization and clinical recovery in both microsphere doses compared with standard intravenous alteplase treatment alone. However, the study was terminated prematurely by the sponsor for administrative reasons coincidentally with the three cases of sICH in the second dose tier. Upon review, these sICH occurred in patients with severe strokes and high blood pressures in violation of rtPA treatment protocol. To date this was the last sonothrombolysis study using microspheres [15].

**Meta-analysis of Clinical Trials of Sonothrombolysis**

A meta-analysis of six randomized and three nonrandomized clinical studies of sonothrombolysis, which included over 400 patients, showed that patients who underwent sonothrombolysis had a 3 times higher chance for complete recanalization and a 2 times higher chance for non-disability after 3 months without increasing the risk of sICH [59]. Cochrane Stroke Group identified five eligible studies with a total of 223 patients [60]. They found that patients (among 206 patients with available data) treated with sonothrombolysis were less likely to be dead or disabled at three months (OR 0.50; 95% CI 0.27-0.91). Failure to recanalize was lower in the sonothrombolysis group (230 patients) (OR 0.28; 95% CI 0.16-0.50). There was no significant difference in cerebral hemorrhage (233 patients). Thus, authors concluded that sonothrombolysis was likely to reduce death or dependency at three months and increase recanalization without clear hazard [60]. A recently published meta-analysis of sonolysis and sonothrombolysis included 10 studies (seven randomized control trials and three case control studies) [61]. Pooled analysis of these studies for a total of 620 patients (345 patients in the treatment group and 275 patients in the control group) showed that patients who received any form of sonothrombolysis (1.8-4 MHz), with or without microspheres, and with or without IV rtPA, had more than twofold higher likelihood of achieving complete recanalization within 1-2 hours from treatment onset in comparison to patients who did not receive any form of sonothrombolysis (OR 2.95; 95% CI 1.81-4.81; p<0.000001). In addition, the patients treated with sonolysis/sonothrombolysis were more likely to achieve a favorable functional outcome (mRS 0-2) at three months (OR 2.20; 95% CI 1.52-3.19; p<0.0001). Sonolysis and sonothrombolysis alone or with microspheres were safer and carried the same risk of sICH as IV rtPA alone (OR 1.14; 95% CI 0.56-2.34; p=0.71).

However, according to the current recommendations, the effectiveness of sonothrombolysis for treatment of patients with acute ischemic stroke is still not well established (Class IIb; Level of Evidence B) [62].
Perspectives for Sonothrombolysis - Operator-independent Device for Sonothrombolysis

The main limitation of TCD technology is the operator-dependency and the need for a qualified sonographer to be present at bedside to find, aim and deliver ultrasound beam to the thrombus residual flow interface [2,63]. Therefore, an operator-independent device for sonothrombolysis is needed to conduct a multicenter, randomized clinical trial [64]. The collaborative group of the CLOTBUST trial first measured the outputs of all devices used [65], then designed multi-transducer assembly to cover conventional windows used for TCD examinations [66]. Further, this novel device was prospectively evaluated for safety in human volunteers [67] and showed that it was well tolerated by stroke-free volunteers and did not cause any neurological dysfunction nor did it affect blood brain barrier integrity. Subsequent phase II study in patients receiving iv rtPA showed that the operator independent device was safe and led to 40% complete MCA recanalization after 2 hours of monitoring [68].

Since this operator-independent, battery-powered device can be quickly mounted by medical personnel with no prior experience in ultrasound, the device enables us to conduct large scale sonothrombolysis trials at all levels of emergency rooms capable of administering rtPA as the standard of care, including telemedicine-supported sites. Thus, sonothrombolysis for acute ischemic stroke is now tested in a pivotal efficacy multi-national trial called CLOTBUSTER (Combined Lysis of ThromBus using 2 MHz pulsed wave Ultrasound and Systemic TPA for Emergent Revascularization, NCT01098981). All patients who will be treated with IV rtPA with NIHSS scores ≥10 points are eligible and they will wear an operator-independent ultrasound emitting device for 2 h. The proprietary device (Cerevast Therapeutics, Redmond, WA) exposes traditional TCD bone windows for sequential insonation of the 12 proximal intracranial segments that most commonly contain thromboembolic occlusions causing disabling strokes. Patients will be randomized 1:1 to continuous exposure to 2 MHz pulsed wave ultrasound versus sham exposure. Safety will be determined by the incidence of symptomatic ICH within 24 hours of treatment. Functional recovery will be determined by mRS at 3 months. CLOTBUSTER is a large simple efficacy clinical trial, the first of its kind for sonothrombolysis. Once CLOTBUSTER establishes safety and efficacy of an operator-independent ultrasound device, the next phase clinical trials can commence combining experimental microspheres with regulatory-approved rtPA therapy and safe ultrasound exposure. This exposure is needed to activate microspheres, however a proof of safety and efficacy of ultrasound is required before a complex combinatory treatment with or without rtPA can be tested any further in the clinical setting [2].

Conclusions

In a quest for stroke treatment, recanalization proved to be the first step to the success. Sonothrombolysis using diagnostic transcranial ultrasound (transcranial Doppler and transcranial color-coded duplex) in combination with recombinant tissue plasminogen activator improves recanalization of an acute intracranial artery occlusion. It can be performed at bedside using commercially available vascular diagnostic ultrasound systems without significant increase in the risk of intracranial hemorrhage. A novel operator-independent device offers a promising solution to avoid the need for an experienced sonographer to be present in the emergency room to deliver sonothrombolysis. Meanwhile, portable diagnostic 2MHz transcranial Doppler or transcranial color-coded duplex are standard equipment in many stroke units, even in developing countries, that can be used for detection of arterial occlusion as well as assessment of recanalization, emboli detection, collateralization of flow with extracranial internal carotid artery disease, alternating flow signals indicative of steal phenomenon. Pending results of ongoing studies, ultrasound remains a promising tool to enhance systemic thrombolysis, and a reliable diagnostic method for the assessment of cerebral hemodynamics in real time.

References


