Sonothrombolysis for Ischemic Stroke

Subjects: Clinical Neurology

Contributor: Narayanaswamy Venketasubramanian , Leonard L. L. Yeo , Benjamin Tan , Bernard P. L. Chan

Stroke is a major cause of death and disability globally, with ischemic stroke being the predominant mechanism. While spontaneous recanalization may occur, significant neuronal injury would have occurred in the interim. Intravenous thrombolysis administered within the first 4.5 h after stroke onset and endovascular thrombectomy within 24 h in patients with a salvageable penumbra improves functional independence. Ultrasound has been shown in both in vivo and in vitro models to enhance clot lysis, even more-so in the presence of thrombolytic agents. The use of transcranial Doppler and transcranial color-coded Doppler ultrasound in acute IS has been reported in case series, case-controlled studies, and clinical trials. While ultrasound at a frequency of 300 kHz increases the risk of intracranial hemorrhage, the 2 MHz range ultrasound aids thrombolysis and improves recanalization without significantly increasing the risk of symptomatic intracranial hemorrhage. Despite this, functional independence was not increased in clinical trials, nor was a benefit shown with the adjunctive use of microbubbles or microspheres. Nonetheless, newer technologies such as endovascular ultrasound, endovascular delivery of microbubbles, and thrombolytic-filled microbubbles await clinical trials. More evidence is needed before sonothrombolysis can be routinely used in the hyperacute management of ischemic stroke.

ischemic stroke sonothrombolysis ultrasound thrombolysis

1. Introduction

Stroke is a major cause of health burden globally. Based on the Global Burden of Disease Study, in 2019, stroke incidence was 12.2 million cases (95% UI 11.0–13.6), with a prevalence of 101 million cases (95% UI 93.2–111) and a mortality of 6.55 million cases (95% UI 6.00–7.02), with 143 million (95% UI 133–153) disability-adjusted life years (DALYs) lost due to stroke [1]. Among incident cases, the most common cause of stroke was arterial occlusion causing ischemic stroke (IS) at 62.4% (7.63 million [95% UI 6.57–8.96]), with 27.9% (3.41 million [95% UI 2.97–3.91]) due to intracerebral hemorrhage (ICH), and 9.7% (1.18 million [95% UI 1.01–1.39]) due to subarachnoid hemorrhage (SAH).

It is estimated that in patients with IS, due to acute large arterial occlusion, in each hour, 120 million neurons, 830 billion synapses, and 714 km of myelinated fibers are lost; in each minute, the respective numbers are 1.9 million, 14 billion, and 12 km [2]. This has bolstered the concept that ‘time is brain’ and the need to begin treatment as soon as possible. Thus, among the key approaches in the management of acute IS is emergent and rapid revascularization of the occluded artery to re-establish adequate perfusion to ischemic tissue so as to rescue as much brain tissue as possible and limit further damage caused by ongoing ischemia [3].

2. Recanalization after Ischemic Stroke

In a systemic review of 10 angiographic studies among patients with IS (*n* = 1130), the authors found that spontaneous recanalization (SR) occurred in approximately 17% of patients in the first 6 to 8 h after stroke onset, with a lack of occlusion seen in 28% to 50% of patients by four days [4]. This shows that some SR occurs naturally after large vessel occlusion, but the proportion is small in the early hours after IS onset, and still poor even after four days, which, in the absence of adequate collateral flow, can lead to brain tissue continuing to be damaged from inadequate perfusion and ongoing ischemia. It would be difficult to obtain similar data on small vessel occlusion due to their tiny size and difficulty in their visualization on cerebral angiography, but as these are usually end-arteries with little collateral flow, the ischemic injury, although to a smaller volume of brain tissue, is likely to be more severe within that territory.

As a corollary, in a study of adults, 38 with IS compared to 17 healthy controls, central motor conduction times (CMCTs) at day 14 post-stroke were improved if spontaneous recanalization occurred within 24 hours compared to after 24 h (87% vs. 62% of patients, $p = 0.005$); CMCT improved in only 17% of those with no recanalization [5]. Thus, early recanalization is crucial. Delayed recanalization, even if it occurs, may not only be unhelpful in aiding stroke recovery due to irrecoverable tissue damage from prolonged inadequate perfusion, but it also carries the serious risk of reperfusion vasogenic oedema and hemorrhagic transformation, which may be deleterious to the already fragile ischemic tissue [6][7].

The process of SR involves the natural lysis of the fibrin network that holds the clot together, allowing blood to flow past the site of occlusion [8]. SR is achieved by plasmin-dependent and plasmin-independent pathways. Of the plasmin-dependent pathways, vascular endothelial cells produce activators of fibrinolysis, such as the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA). These activators convert circulating plasminogen to plasmin, which then breaks down fibrin and lyses the clot. Plasmin-independent pathways involve the accumulation of polymorphonuclear leucocytes at the site of the clot that then activate plasmin-dependent pathways as well as release serine proteases, including proteinase 3, cathepsin G, and elastase, that break up the clot. Monocytes infiltrating into the clot also release plasminogen activators that then cause clot lysis.

Various thrombolytic agents have been developed that are able to enhance the intrinsic clot lysis by their ability to convert circulating plasminogen to plasmin, which then lyses the clot [9]. Such agents include alteplase, duteplase, desmoteplase, reteplase, staphylokinase, streptokinase, pro-urokinase, urokinase, tenecteplase, and anistreplase (APSAC), to name a few. In a meta-analysis of 27 randomized trials (*n* = 10,187), thrombolytic therapy, mostly given up to six hours after the onset of IS, significantly reduced the odds of death or dependency (modified Rankin Score mRS 3–6) at three to six months after a stroke (odds ratio (OR) 0.85, 95%CI 0.78–0.93) [10]). When administered within three hours of the onset of IS (11 trials, *n* = 2187), death or dependency was even more significantly reduced (OR 0.66, 95% CI 0.56–0.79) without increasing mortality. The early administration of intravenous thrombolysis (IVT) within 4.5 h of acute IS onset among patients without contraindications is now the

standard of care [3]. Patients treated with IVT from 4.5 to 9 h after stroke onset, or wake-up stroke, would also benefit if they had imaging evidence of a perfusion mismatch [11].

Mechanical (also called endovascular) thrombectomy (EVT) involves the physical removal of clots in large intracranial arteries using a device passed intra-arterially to the level of the clot in a technique that uses cerebral angiography to visualize the vasculature. A meta-analysis of five large landmark trials of acute IS patients with occlusion of the proximal anterior circulation artery treated by EVT within 12 h of onset (n = 1287) showed that EVT significantly reduced disability as measured by the mRS at 90 days compared with a control (adjusted cOR 2.49, 95% CI 1.76–3.53) [12]. In a meta-analysis of nine randomized controlled trials combining IVT with EVT (n = 3740) versus EVT alone, the combination group was superior in functional independence (mRS 0–2) (OR 1.27, 95% CI 1.11–1.46) [13]. In a systematic review and meta-analysis of 16 studies (n = 7572) where intra-arterial thrombolysis was added to EVT, functional independence (mRS 0–2) at 90 days was non-significantly different (OR 1.14, 95%CI: 0.95–1.37) [14]. Thrombectomy within 24 h of onset among stroke patients with proximal large artery occlusion and a salvageable penumbra, with bridging IVT if it can be initiated within 4.5 h, is now the standard of care [3]. EVT has shown a functional benefit even among those with significant brain damage, as evidenced by an Alberta Stroke Program Early Computed Tomography Score of 3–5 or a calculated infarct volume of >50 mL [15].

3. Ultrasound

Sound is a form of mechanical energy [16]. Sound occurs when its source vibrates and produces cyclical oscillations of longitudinal waves that then allow the propagation of the energy. There are two phases in a sound wave—compression, which is the high-pressure phase of a sound wave, and rarefaction, which is the low-pressure phase. Sound needs a medium for it to travel—it is unable to travel in a vacuum. The frequency of an ultrasound is the number of wave cycles per second produced by the source—it is expressed in hertz (Hz). Sound waves conducted at a frequency above the upper limit of human hearing of 20 kHz (i.e., above 20,000 Hz) are called ultrasound. Ultrasound frequencies for clinical use range from 1 MHz to 20 MHz (i.e., 1 million to 20 million Hz).

When ultrasound enters tissue, a small amount is reflected by highly reflective objects back to the source/transducer, which forms the basis of diagnostic ultrasound imaging [16]. The sound waves may also be refracted if they pass through a different medium and thus shift direction. They may be scattered in multiple directions, or they may be absorbed, which forms the basis of therapeutic ultrasound. The absorbed energy causes vibration, heating, and the occurrence of vapor- or gas-filled cavitations/bubbles, as well as circulation of the liquid and the appearance of oxidation products [17].

Cavitation occurs when ultrasound passing through a liquid medium interacts with gaseous inclusions in that medium, e.g., microbubbles (MBs) [18]. Cavitation is broadly divided into stable cavitation (also called gas body activation (formerly identified as stable cavitation)), and inertial cavitation (also called transient cavitation). In stable cavitation, a relatively low level of ultrasound intensity activates a pre-existing gas body that resonates and undergoes periodic and regular volume changes in response to the applied acoustic pressure. Inertial cavitation requires a relatively higher ultrasound intensity that makes the MBs also undergo periodic volume changes in sync

with the applied acoustic pressure, but the rapid increases in size lead to the bubble becoming unstable and then violently imploding.

4. In Vitro and In Vivo Effects of Ultrasound on Clots

The effect of ultrasound on blood clots has long been studied, with laboratory evidence in the 1950s showing that it is able to compress and retract clots [19][20]. While high-intensity ultrasound is able to mechanically fragment clots [21], low-intensity ultrasound is able to augment enzymatic fibrinolysis through non-thermal mechanisms by improving entry of fibrinolysis activators into the clot [22], reversibly altering fibrin structure and increasing tPA binding to fibrin [23]. The main mechanisms for clot lysis by ultrasound include acoustic stable cavitation and radiation force [24]. The use of ultrasound to cause or enhance clot lysis is called ‘sonothrombolysis’.

Among the earliest studies of therapeutic ultrasound for clot lysis, Sobbe et al. [25], building on earlier work by Ehringer et al. [26], showed that ultrasound was able to recanalize thrombosed femoral arteries in dogs without complications. Transcutaneous ultrasound applied in vivo combined with streptokinase was able to significantly augment the lysis of thrombi in the iliofemoral arteries of rabbits [27].

High-intensity focused ultrasound (1.5 MHz) initiated thrombolysis effectively and safely in a rabbit model of embolic stroke [28]. Ultrasound combined with a recombinant tissue plasminogen activator (rTPA) was more effective than rTPA treatment alone in reducing infarct volume in an embolic rat stroke model [29].

The value of magnetic resonance imaging (MRI)-guided focused ultrasound (MRgFUS) has been investigated. In a study using MRgFUS and monitoring using an MR angiogram (MRA) performed at 1-minute intervals, a 1 MHz ultrasound combined with rTPA was better able than rTPA alone to dissolve clots in the carotid artery of New Zealand rabbits [30].

Studies also evaluated the MRgFUS system through a plastic phantom skull. When combined with thrombolytic drugs and using transducers at either 0.5 or 1 MHz, clots could be destroyed in this in-vitro model [31].

In a study using a human temporal bone, a 1.8-MHz pulsed-wave (PW) ultrasound was passed through a human temporal bone onto whole blood clots. While clot weight was reduced using ultrasound alone compared to controls, an even greater reduction was achieved when ultrasound was combined with rTPA compared with rTPA alone [32].

To explore the impact of the human calvarium, artificial thrombi were placed inside the skull and located at the transducer focus point. Clot lysis increased with increasing acoustic output powers [33]. In another study, longer duty cycles combined with longer pulse widths had the highest potential to lyse clots [34].

Lower-frequency ultrasound in the kHz range, compared to the MHz range, is less attenuated by bone and is thus able to pass through the skull more easily [35]. Embolic rat models have shown that low-frequency ultrasound is

able to significantly reduce infarct volume compared to pure TPA treatment. Animal studies did not show an increased rate of bleeding or harm to the blood–brain barrier.

There are reasonable concerns as to the formation of clot fragments when the clot is broken down by ultrasound, leading to the risk of distal embolization of these fragments and subsequent vascular occlusion. Rosenschein et al. showed in an animal model that 93% of clot fragments from sonothrombolysis were sub-capillary in size (<8 microns) [36] and thus unlikely to cause significant distal vascular occlusion. Other researchers have also reported very small fragments, 96% being <5 microns [37] or all being <11 microns [38]. It can be expected that the body's endogenous fibrinolytic system would dissolve fragments, a process further enhanced by the co-administration of thrombolytic agents.

5. Microbubbles

Further progress came with the increased understanding of MBs. Under conditions of inertial cavitation, due to Bjerknes acoustic radiation forces and large-amplitude oscillations of MBs, there is a mechanical effect causing thrombus pitting and deformation [39]. The expansion and collapse of bubbles are able to stretch individual fibers within clots, while the dissipation of acoustic energy is able to break individual fibers in the clot—if the bubble located outside the clot collapses asymmetrically, an impinging jet is created that aids in clot destruction [40].

MB-mediated sonothrombolysis is a complex process involving the clot, ultrasound, thrombolytic drug, and MBs. Clot lysis is best achieved by combining ultrasound at a high acoustic pressure with TPA and MB, compared to a single agent or in any other combination [41]. A similar result was seen an in vitro circulating flow model evaluating the role of tenecteplase (TNK-tPA)-mediated thrombolysis of fully retracted porcine blood clots where higher FUS frequencies (1 MHz) are associated with better thrombolysis compared to lower FUS frequencies (0.6 MHz), and an even better thrombolytic efficacy of the combination of 1-MHz FUS pulses with TNK-tPA and MBs [42]. In a thromboembolic stroke model of middle cerebral artery occlusion (MCAO) in rats, MB-mediated sonothrombolysis led to a similar reduction in infarct volume compared to rTPA [43]. A systematic review of 16 pre-clinical studies found heterogeneity in ultrasound parameters and types of MBs used, but four studies showed the superiority of sonothrombolysis over rTPA based on clinical criteria; however, safety data was limited [44].

Contrast agents are MBs that are given intravenously, are sufficiently small and stable enough to survive the cardiopulmonary circulation, and remain in the circulation to then enter other vascular beds, e.g., the cerebral circulation [45]. These MBs increase the Doppler signal by 10 dB to 30 dB, allowing the detection of flow in locations for which attenuation may make successful insonation impossible, e.g., the transcranial examination of intracranial vessels, especially through unfavorable bone windows. They can increase the success rate of the examination, reduce the time required for the examination, and facilitate the detection of a greater number of vessels.

Contrast agents and MB formulations used in animal models include Sonovue, Definity, perflutren lipoid, albumin, and BR38 [46][47][48][49][50]. Magnetic targeting of MBs using a single magnet allows for increased lysis rates [51].

New research has shown that different shapes showed different cavitation reactivity, e.g., of two different gold nanoparticles in shape functionalized with the rtPA, rtPA-functionalized asymmetric gold nanostars (NSt) were superior to gold nanospheres (NPt) in causing cavitation [52]. As clots fragmented by sonothrombolysis may result in debris that then migrates distally and causes further occlusions, phase-change nanodroplets can reduce the overall clot debris size and may allow for safer sonothrombolysis [53].

More recent techniques involve the intraluminal visualization of clots and the delivery of ultrasound intravascularly [54]. Intra-arterial B-mode ultrasound imaging was able to verify intra-clot delivery and clot penetration of the concurrently administered ultrasound contrast agents Optison and SonoVue with rTPA [55]. MBs may also be delivered intra-arterially while the ultrasound is applied from an extracranial source [56]. A dual-mode ultrasound catheter that combined a 16-MHz high-frequency element (imaging transducer) and a 220-kHz low-frequency element (treatment transducer) showed successful in-vitro sonothrombolysis [57].

Hollow nanogels loaded with thrombolytic agents, e.g., urokinase-type plasminogen activator (uPA), octafluoropropane, and rTPA-loaded echogenic liposomes (OFP t-ELIP), when sonolysed at the site of the clot, allow the delivery of thrombolytic agents at the site of the thrombus [58].

In parallel, there has been a greater understanding of how the characteristics of a clot may affect its susceptibility to sonothrombolysis. Sonothrombolysis is more successful on fresh thrombi and those with high cholesterol levels within it [59]. Platelet-rich clots are resistant to thrombolysis, which may explain some of the failures in recanalization [60]. Higher acoustic pressure is needed to lyse arterial thrombi compared to venous thrombi [61].

6. Transcranial Ultrasound Imaging

The intracranial structures can be imaged in various ways, including by computed tomography (CT) and MRI [62]. The vasculature can be studied by CT angiography (CTA), where intravenous contrast is injected and high-resolution small-thickness slice images are rapidly taken of the brain and blood flow through the vessels and usually reconstructed into easily interpretable images—while a rapid technique and widely available, it does carry a radiation risk and needs a contrast injection which can be an issue in those with renal dysfunction or a contrast allergy. MR angiography (MRA) is another method where the signals generated by flowing blood in a magnetic field are used to again reconstruct easily interpretable images of blood flowing through the vessels—while it carries no radiation risk and does not usually require contrast injection, it may not be easily available especially after office hours if at all, takes much longer than CTA, and may be a challenge in claustrophobic or clinically unstable patients, or those with pacemakers.

Ultrasound imaging has the advantages of being safe, portable, and rapid, and is usually widely available—transcranial Doppler (TCD) is routinely performed on stroke centers to evaluate blood flow in the basal cerebral arteries in acute stroke [63]. Transcranial color-coded duplex (TCCD) imaging marries the blind TCD technique with B-mode imaging and color coding of Doppler signals generated by flowing to allow a more accurate ultrasound visualization of the intracranial vessels and is also used in stroke centers as a complementary technique to study

blood flow [64]. Both TCD and TCCD involve passing low-frequency ultrasound waves in the 2 MHz range through the intact skull to visualize flowing blood and can diagnose arterial stenosis, vasospasm, occlusion, compensatory flow, and embolization, to name a few [65].

7. Clinical Case Series

There have been a few publications reporting the clinical experience with sonothrombolysis for acute ischemic stroke.

8. Case-Controlled Studies and Non-Randomized Clinical Trials

While case series are able to provide useful initial information, comparing with suitable controls would allow a more accurate understanding of outcomes.

9. Randomized Controlled Trials

Adequately powered randomized controlled trials (RCTs) are considered the gold standard to assess the efficacy and safety of medical interventions. There have been a number of RCTs of sonothrombolysis for acute stroke published since 2003.

10. Progress in Ultrasound Delivery to Patients

The traditional ultrasound beam at a frequency of 2 MHz allows for the insonation of deeper structures but suffers from severe attenuation by the skull and the highly demanding skill needed to focus the beam at the site of occlusion [63]. The initial promise of an easy-to-use device using a low frequency of 300 kHz that would transgress the skull easily and not need precise targeting of the occlusion [33] was broken when the TRUMBI trial showed an unacceptable risk of ICH [66]. Progress was made when a hands-free, operator-independent device was developed and shown to be safe before being tested in the CLOTBUST-ER clinical trial [67].

Delivery of ultrasound intra-arterially was tested using the EkoSonic Endovascular System in a prospective study of 14 patients, mean age 65.1 ± 11.2 years, with moderate acute IS and occluded middle cerebral artery (n = 7) or basilar artery (n = 7), compared with historical controls [68]. Endovascular sonolysis was started within 8 h of stroke onset. Arterial recanalization was achieved in 85.7% of patients with MCA occlusion and 100% with BA occlusion. There were no symptomatic intracerebral hemorrhage nor periprocedural complications. The median mRS at 90 days was one (IQR 1–3.5) in MCA occlusion and three (IQR 2–4.5) in BA occlusion, compared to five (IQR 305) and six (IQG4.5–6), respectively, among the controls.

11. Stroke Prevention

In view of the risk of new brain ischemic lesions that may occur in up to two-thirds of patients undergoing coronary artery bypass grafting or cardiac valve surgery, Skoloudik et al. found in the randomized controlled SONORESCUE trial (n = 60 vs. 60, mean age 65.3 yr) that intra-operative sonolysis would reduce the risk of new lesions > 0.5 mL as well as the median volume of new ischemic lesions on diffusion-weighted imaging (DWI) MRI in this group of patients [69]. Clinical stroke or transient ischemic attack (TIA) occurred in 0% of treated vs. 3.3% of untreated patients (p = 0.496). The subsequent SONOREDUCE randomized controlled trial involving 144 patients undergoing coronary stenting only was neutral [70].

As silent brain infarctions may occur in up to one-third of patients undergoing carotid endarterectomy and two-thirds of those undergoing carotid angioplasty and/or stenting, Skoloudik et al. investigated in the randomized controlled SONOBUSTER trial the use of intraoperative sonolysis in these patients [71]. They found that new lesions on an MRI were again less frequent among the 121 patients (mean age, 66.65 ± 7.17 years) compared to 121 controls (75; 66.02 ± 8.11 years). Clinical stroke or TIA occurred in 0.8% vs. 2.5% respectively (p > 0.3).

In the randomized controlled SONOBIRDIE trial of sonolysis of patients while they were undergoing carotid endarterectomy, (n = 1004, mean age 68 ± 7.8 years), the primary composite outcome of ischemic stroke, TIA, or death within 30 days occurred in 2.2% vs. 7.6% in controls (risk difference 5.5%; 95% CI 2.8–8.3%; p < 0.001)—stroke/TIA occurred in 1.8% vs. 7.5% (p < 0.001) while a new ischemic lesion on an MRI was seen in 8.6% vs. 17.4% of patients (p < 0.01) [72].

12. Conclusions

Emergent recanalization of occluded arteries remains the holy grail of hyperacute therapy in ischemic stroke. IVT and, more recently, EVT for carefully selected patients are now the standard of care. Transcranial ultrasound either as TCD or TCCD, alone or in addition to IVT, for occluded arteries, in the 2 MHz range, has been shown in vitro and in vivo, both in animal models and humans, to aid thrombolysis and improve recanalization without significantly increasing the risk of symptomatic intracranial hemorrhage. The use of 300 kHz ultrasound is unsafe. The adjunctive use of MBs or microspheres has not shown a benefit in randomized controlled trials. Further evidence is needed on the clinical value of endovascular ultrasound. Other techniques explored in animal models, including the endovascular delivery of MBs, the use of IVTMBs filled with thrombolytics, and differentially shaped nanoparticles, await clinical trials. While much has been done, much more lies ahead as evidence is generated for the clinical role of sonothrombolysis in the management of acute ischemic stroke.

References

1. GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol. 2021, 20, 795–820.

2. Saver, J.L. Time is brain—Quantified. Stroke 2006, 37, 263–266.
3. Powers, W.J.; Rabinstein, A.A.; Ackerson, T.; Adeoye, O.M.; Bambakidis, N.C.; Becker, K.; Biller, J.; Brown, M.; Demaerschalk, B.M.; Hoh, B.; et al. Guidelines for the Early Management of Patients with Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. Stroke 2019, 50, e344–e418.
4. Kassem-Moussa, H.; Graffagnino, C. Nonocclusion and spontaneous recanalization rates in acute ischemic stroke: A review of cerebral angiography studies. Arch. Neurol. 2002, 59, 1870–1873.
5. Vang, C.; Dunbabin, D.; Kilpatrick, D. Effects of spontaneous recanalization on functional and electrophysiological recovery in acute ischemic stroke. Stroke 1999, 30, 2119–2125.
6. Camara, R.; Matei, N.; Zhang, J.H. Evolution of the stroke paradigm: A review of delayed recanalization. J. Cereb. Blood Flow Metab. 2021, 41, 945–957.
7. Gourdin, M.J.; Bree, B.; De Kock, M. The impact of ischaemia-reperfusion on the blood vessel. Eur. J. Anaesthesiol. 2009, 26, 537–547.
8. Kovacs, I.B.; Yamamoto, J. Spontaneous thrombolysis: A forgotten determinant of life or death. Clin. Appl. Thromb. Hemost. 2006, 12, 358–363.
9. Mican, J.; Toul, M.; Bednar, D.; Damborsky, J. Structural Biology and Protein Engineering of Thrombolytics. Comput. Struct. Biotechnol. J. 2019, 17, 917–938.
10. Wardlaw, J.M.; Murray, V.; Berge, E.; del Zoppo, G.J. Thrombolysis for acute ischaemic stroke. Cochrane Database Syst. Rev. 2014, 2014, CD000213.
11. Berge, E.; Whiteley, W.; Audebert, H.; De Marchis, G.M.; Fonseca, A.C.; Padiglioni, C.; de la Ossa, N.P.; Strbian, D.; Tsivgoulis, G.; Turc, G. European Stroke Organisation (ESO) guidelines on intravenous thrombolysis for acute ischaemic stroke. Eur. Stroke J. 2021, 6, I–LXII.
12. Goyal, M.; Menon, B.K.; van Zwam, W.H.; Dippel, D.W.; Mitchell, P.J.; Demchuk, A.M.; Dávalos, A.; Majoie, C.B.; van der Lugt, A.; de Miquel, M.A.; et al. Endovascular thrombectomy after large-vessel ischaemic stroke: A meta-analysis of individual patient data from five randomised trials. Lancet 2016, 387, 1723–1731.
13. Zheng, M.; Li, L.; Chen, L.; Li, B.; Feng, C. Mechanical thrombectomy combined with intravenous thrombolysis for acute ischemic stroke: A systematic review and meta-analyses. Sci. Rep. 2023, 13, 8597.
14. Qureshi, A.I.; Lodhi, A.; Akhtar, I.N.; Ma, X.; Kherani, D.; Kwok, C.S.; Ford, D.E.; Hanley, D.F.; Hassan, A.E.; Nguyen, T.N.; et al. Mechanical thrombectomy with intra-arterial thrombolysis versus mechanical thrombectomy alone in patients with acute ischemic stroke: A systematic review and meta-analysis. Int. J. Stroke 2024, 19, 16–28.

15. Atchley, T.J.; Estevez-Ordonez, D.; Laskay, N.M.B.; Tabibian, B.E.; Harrigan, M.R. Endovascular Thrombectomy for the Treatment of Large Ischemic Stroke: A Systematic Review and Meta-Analysis of Randomized Control Trials. Neurosurgery 2024, 94, 29–37.
16. Grogan, S.P.; Mount, C.A. Ultrasound Physics and Instrumentation. . In StatPearls ; StatPearls Publishing: Treasure Island, FL, USA, 2023. Available online: https://www.ncbi.nlm.nih.gov/books/NBK570593/ (accessed on 13 February 2024).
17. Goldman, D.E.; Lepeschkin, W.W. Injury to living cells in standing sound waves. J. Cell Comp. Physiol. 1952, 40, 255–268.
18. Miller, M.W.; Miller, D.L.; Brayman, A.A. A review of in vitro bioeffects of inertial ultrasonic cavitation from a mechanistic perspective. Ultrasound Med. Biol. 1996, 22, 1131–1154.
19. Burstein, M.; Lewi, S. Action des ultrasons sur le caillot plasmatique . C. R. Seances Soc. Biol. Fil. 1951, 145, 1599–1600.
20. Burstein, M.; Lewi, S. Sur les propriétés physiques du caillot plasmatique; compressibilité et rétraction par ultrasons . C. R. Seances Soc. Biol. Fil. 1952, 146, 1544–1546.
21. Howkins, S.D.; Weinstock, A. The effect of focused ultrasound on human blood. Ultrasonics 1970, 8, 174–176.
22. Francis, C.W.; Suchkova, V.N. Ultrasound and thrombolysis. Vasc. Med. 2001, 6, 181–187.
23. Francis, C.W. Ultrasound-enhanced thrombolysis. Echocardiography 2001, 18, 239–246.
24. Bader, K.B.; Gruber, M.J.; Holland, C.K. Shaken and stirred: Mechanisms of ultrasound-enhanced thrombolysis. Ultrasound Med. Biol. 2015, 41, 187–196.
25. Sobbe, A.; Stumpff, U.; Trübestein, G.; Figge, H.; Kozuschek, W. Die Ultraschall Auflösung von Thromben . Klin. Wochenschr. 1974, 52, 1117–1121.
26. Ehringer, H.; Fischer, M. Erfolgreiche thrombolytische Therapie bei subakuten arteriellen Thrombosen. Vorläufiger Bericht. . Med. Welt. 1968, 32, 1726–1728.
27. Luo, H.; Nishioka, T.; Fishbein, M.C.; Cercek, B.; Forrester, J.S.; Kim, C.J.; Berglund, H.; Siegel, R.J. Transcutaneous ultrasound augments lysis of arterial thrombi in vivo. Circulation 1996, 94, 775–778.
28. Burgess, A.; Huang, Y.; Waspe, A.C.; Ganguly, M.; Goertz, D.E.; Hynynen, K. High-intensity focused ultrasound (HIFU) for dissolution of clots in a rabbit model of embolic stroke. PLoS ONE 2012, 7, e42311.
29. Daffertshofer, M.; Huang, Z.; Fatar, M.; Popolo, M.; Schroeck, H.; Kuschinsky, W.; Moskowitz, M.A.; Hennerici, M.G. Efficacy of sonothrombolysis in a rat model of embolic ischemic stroke. Neurosci. Lett. 2004, 361, 115–119.

30. Damianou, C.; Hadjisavvas, V.; Mylonas, N.; Couppis, A.; Ioannides, K. MRI-guided sonothrombolysis of rabbit carotid artery. J. Stroke Cerebrovasc. Dis. 2014, 23, e113–e121.
31. Damianou, C.; Hadjisavvas, V.; Ioannides, K. In Vitro and In Vivo evaluation of a magnetic resonance imaging-guided focused ultrasound system for dissolving clots in combination with thrombolytic drugs. J. Stroke Cerebrovasc. Dis. 2014, 23, 1956–1964.
32. Eggers, J.; Ossadnik, S.; Seidel, G. Enhanced clot dissolution in vitro by 1.8-MHz pulsed ultrasound. Ultrasound Med. Biol. 2009, 35, 523–526.
33. Wright, C.C.; Hynynen, K.; Goertz, D.E. Pulsed focused ultrasound-induced displacements in confined in vitro blood clots. IEEE Trans. Biomed. Eng. 2012, 59, 842–851.
34. Hölscher, T.; Raman, R.; Fisher, D.J.; Ahadi, G.; Zadicario, E.; Voie, A. Effects of varying duty cycle and pulse width on high-intensity focused ultrasound (HIFU)-induced transcranial thrombolysis. J. Ther. Ultrasound 2013, 1, 18.
35. Daffertshofer, M.; Hennerici, M.G. Sonothrombolysis: Experimental evidence. Front. Neurol. Neurosci. 2006, 21, 140–149.
36. Rosenschein, U.; Furman, V.; Kerner, E.; Fabian, I.; Bernheim, J.; Eshel, Y. Ultrasound imaging-guided noninvasive ultrasound thrombolysis: Preclinical results. Circulation 2000, 102, 238–245.
37. Maxwell, A.D.; Cain, C.A.; Duryea, A.P.; Yuan, L.; Gurm, H.S.; Xu, Z. Noninvasive thrombolysis using pulsed ultrasound cavitation therapy—Histotripsy. Ultrasound Med. Biol. 2009, 35, 1982–1994.
38. Ahadi, G.; Welch, C.S.; Grimm, M.J.; Fisher, D.J.; Zadicario, E.; Ernström, K.; Voie, A.H.; Hölscher, T. Transcranial sonothrombolysis using high-intensity focused ultrasound: Impact of increasing output power on clot fragmentation. J. Ther. Ultrasound 2013, 1, 22.
39. Chen, X.; Leeman, J.E.; Wang, J.; Pacella, J.J.; Villanueva, F.S. New insights into mechanisms of sonothrombolysis using ultra-high-speed imaging. Ultrasound Med. Biol. 2014, 40, 258–262.
40. Weiss, H.L.; Selvaraj, P.; Okita, K.; Matsumoto, Y.; Voie, A.; Hoelscher, T.; Szeri, A.J. Mechanical clot damage from cavitation during sonothrombolysis. J. Acoust. Soc. Am. 2013, 133, 3159–3175.
41. Petit, B.; Bohren, Y.; Gaud, E.; Bussat, P.; Arditi, M.; Yan, F.; Tranquart, F.; Allémann, E. Sonothrombolysis: The contribution of stable and inertial cavitation to clot lysis. Ultrasound Med. Biol. 2015, 41, 1402–1410.
42. Papadopoulos, N.; Yiallouras, C.; Damianou, C. The Enhancing Effect of Focused Ultrasound on TNK-Tissue Plasminogen Activator-Induced Thrombolysis Using an In Vitro Circulating Flow Model. J. Stroke Cerebrovasc. Dis. 2016, 25, 2891–2899.
43. Braun, T.; Sünner, L.; Hachenberger, M.; Müller, C.; Wietelmann, A.; Juenemann, M.; Pons-Kühnemann, J.; Kaps, M.; Gerriets, T.; Tschernatsch, M.; et al. Microbubble-mediated

sonothrombolysis with BR38 of a venous full blood thrombus in a rat embolic stroke model. Ann. Transl. Med. 2021, 9, 1061.

44. Auboire, L.; Sennoga, C.A.; Hyvelin, J.M.; Ossant, F.; Escoffre, J.M.; Tranquart, F.; Bouakaz, A. Microbubbles combined with ultrasound therapy in ischemic stroke: A systematic review of in-vivo preclinical studies. PLoS ONE 2018, 13, e0191788.
45. Burns, P.N. Overview of echo-enhanced vascular ultrasound imaging for clinical diagnosis in neurosonology. J. Neuroimaging 1997, 7 (Suppl. 1), S2–S14.
46. Nedelmann, M.; Ritschel, N.; Doenges, S.; Langheinrich, A.C.; Acker, T.; Reuter, P.; Yeniguen, M.; Pukropski, J.; Kaps, M.; Mueller, C.; et al. Combined contrast-enhanced ultrasound and rt-PA treatment is safe and improves impaired microcirculation after reperfusion of middle cerebral artery occlusion. J. Cereb. Blood Flow Metab. 2010, 30, 1712–1720.
47. Brown, A.T.; Flores, R.; Hamilton, E.; Roberson, P.K.; Borrelli, M.J.; Culp, W.C. Microbubbles improve sonothrombolysis in vitro and decrease hemorrhage in vivo in a rabbit stroke model. Investig. Radiol. 2011, 46, 202–207.
48. Pajek, D.; Burgess, A.; Huang, Y.; Hynynen, K. High-intensity focused ultrasound sonothrombolysis: The use of perfluorocarbon droplets to achieve clot lysis at reduced acoustic power. Ultrasound Med. Biol. 2014, 40, 2151–2161.
49. Culp, W.C.; Flores, R.; Brown, A.T.; Lowery, J.D.; Roberson, P.K.; Hennings, L.J.; Woods, S.D.; Hatton, J.H.; Culp, B.C.; Skinner, R.D.; et al. Successful microbubble sonothrombolysis without tissue-type plasminogen activator in a rabbit model of acute ischemic stroke. Stroke 2011, 42, 2280–2285.
50. Schleicher, N.; Tomkins, A.J.; Kampschulte, M.; Hyvelin, J.M.; Botteron, C.; Juenemann, M.; Yeniguen, M.; Krombach, G.A.; Kaps, M.; Spratt, N.J.; et al. Sonothrombolysis with BR38 Microbubbles Improves Microvascular Patency in a Rat Model of Stroke. PLoS ONE 2016, 11, e0152898.
51. de Saint Victor, M.; Barnsley, L.C.; Carugo, D.; Owen, J.; Coussios, C.C.; Stride, E. Sonothrombolysis with Magnetically Targeted Microbubbles. Ultrasound Med. Biol. 2019, 45, 1151–1163.
52. Choi, W.; Key, J.; Youn, I.; Lee, H.; Han, S. Cavitation-assisted sonothrombolysis by asymmetrical nanostars for accelerated thrombolysis. J. Control Release 2022, 350, 870–885.
53. Guo, S.; Guo, X.; Wang, X.; Zhou, D.; Du, X.; Han, M.; Zong, Y.; Wan, M. Reduced clot debris size in sonothrombolysis assisted with phase-change nanodroplets. Ultrason. Sonochem. 2019, 54, 183–191.
54. Wu, H.; Tang, Y.; Zhang, B.; Klippel, P.; Jing, Y.; Yao, J.; Jiang, X. Miniaturized Stacked Transducer for Intravascular Sonothrombolysis with Internal-Illumination Photoacoustic Imaging

Guidance and Clot Characterization. IEEE Trans. Biomed. Eng. 2023, 70, 2279–2288.

55. Soltani, A.; Singhal, R.; Obtera, M.; Roy, R.A.; Clark, W.M.; Hansmann, D.R. Potentiating intra-arterial sonothrombolysis for acute ischemic stroke by the addition of the ultrasound contrast agents (Optison™ & SonoVue(®)). J. Thromb. Thrombolysis 2011, 31, 71–84.
56. Dixon, A.J.; Li, J.; Rickel, J.R.; Klibanov, A.L.; Zuo, Z.; Hossack, J.A. Efficacy of Sonothrombolysis Using Microbubbles Produced by a Catheter-Based Microfluidic Device in a Rat Model of Ischemic Stroke. Ann. Biomed. Eng. 2019, 47, 1012–1022.
57. Wu, H.; Zhang, B.; Huang, C.C.; Peng, C.; Zhou, Q.; Jiang, X. Ultrasound-Guided Intravascular Sonothrombolysis with a Dual Mode Ultrasound Catheter: In Vitro Study. IEEE Trans. Ultrason. Ferroelectr. Freq. Control. 2022, 69, 1917–1925.
58. Shekhar, H.; Kleven, R.T.; Peng, T.; Palaniappan, A.; Karani, K.B.; Huang, S.; McPherson, D.D.; Holland, C.K. In vitro characterization of sonothrombolysis and echocontrast agents to treat ischemic stroke. Sci. Rep. 2019, 9, 9902.
59. Zhou, Y.; Murugappan, S.K.; Sharma, V.K. Effect of clot aging and cholesterol content on ultrasound-assisted thrombolysis. Transl. Stroke Res. 2014, 5, 627–634.
60. Tomkins, A.J.; Schleicher, N.; Murtha, L.; Kaps, M.; Levi, C.R.; Nedelmann, M.; Spratt, N.J. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. Exp. Transl. Stroke Med. 2015, 7, 2.
61. Black, J.J.; Yu, F.T.; Schnatz, R.G.; Chen, X.; Villanueva, F.S.; Pacella, J.J. Effect of Thrombus Composition and Viscosity on Sonoreperfusion Efficacy in a Model of Micro-Vascular Obstruction. Ultrasound Med. Biol. 2016, 42, 2220–2231.
62. Regenhardt, R.W.; Potter, C.A.; Huang, S.S.; Lev, M.H. Advanced Imaging for Acute Stroke Treatment Selection: CT, CTA, CT Perfusion, and MR Imaging. Radiol. Clin. N. Am. 2023, 61, 445–456.
63. Wan, Y.; Teng, X.; Li, S.; Yang, Y. Application of transcranial Doppler in cerebrovascular diseases. Front. Aging Neurosci. 2022, 14, 1035086.
64. Baumgartner, R.W.; Mattle, H.P.; Aaslid, R. Transcranial color-coded duplex sonography, magnetic resonance angiography, and computed tomography angiography: Methods, applications, advantages, and limitations. J. Clin. Ultrasound 1995, 23, 89–111.
65. Sloan, M.A.; Alexandrov, A.V.; Tegeler, C.H.; Spencer, M.P.; Caplan, L.R.; Feldmann, E.; Wechsler, L.R.; Newell, D.W.; Gomez, C.R.; Babikian, V.L.; et al. Assessment: Transcranial Doppler ultrasonography: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 2004, 62, 1468–1481.

66. Daffertshofer, M.; Gass, A.; Ringleb, P.; Sitzer, M.; Sliwka, U.; Els, T.; Sedlaczek, O.; Koroshetz, W.J.; Hennerici, M.G. Transcranial low-frequency ultrasound-mediated thrombolysis in brain ischemia: Increased risk of hemorrhage with combined ultrasound and tissue plasminogen activator: Results of a phase II clinical trial. Stroke 2005, 36, 1441–1446.
67. Alexandrov, A.V.; Köhrmann, M.; Soinne, L.; Tsivgoulis, G.; Barreto, A.D.; Demchuk, A.M.; Sharma, V.K.; Mikulik, R.; Muir, K.W.; Brandt, G.; et al. Safety and efficacy of sonothrombolysis for acute ischaemic stroke: A multicentre, double-blind, phase 3, randomised controlled trial. Lancet Neurol. 2019, 18, 338–347.
68. Kuliha, M.; Roubec, M.; Jonszta, T.; Krajca, J.; Czerny, D.; Krajina, A.; Langová, K.; Herzig, R.; Procházka, V.; Školoudík, D. Safety and efficacy of endovascular sonolysis using the EkoSonic endovascular system in patients with acute stroke. AJNR Am. J. Neuroradiol. 2013, 34, 1401–1406.
69. Školoudík, D.; Hurtíková, E.; Brát, R.; Herzig, R.; SONORESCUE Trial Group. Sonolysis in Prevention of Brain Infarction During Cardiac Surgery (SONORESCUE): Randomized, Controlled Trial. Medicine 2016, 95, e3615.
70. Viszlayová, D.; Brozman, M.; Langová, K.; Herzig, R.; Školoudík, D.; a SONOREDUCE Trial Group. Sonolysis in risk reduction of symptomatic and silent brain infarctions during coronary stenting (SONOREDUCE): Randomized, controlled trial. Int. J. Cardiol. 2018, 267, 62–67.
71. Školoudík, D.; Kuliha, M.; Hrbáč, T.; Jonszta, T.; Herzig, R.; SONOBUSTER Trial Group. Sonolysis in Prevention of Brain Infarction During Carotid Endarterectomy and Stenting (SONOBUSTER): A randomized, controlled trial. Eur. Heart J. 2016, 37, 3096–3102.
72. Skoloudik, D.; Hrbáč, T.; Herzig, R.; Fiedler, J.; Beneš, V.; Kesnerova, P.; Kovar, M.; Vosko, M.; Nosal, V.; Beneš, V.; et al. Sonolysis in prevention of brain infarctions during internal carotid endarterectomy (SONOBIRDIE): The results of randomized controlled trial. Eur. Stroke J. 2023, 8, 680.