Comparison of Sonothrombolysis Efficiencies of Different Ultrasound Systems

Yufeng Zhou, PhD,* and Rajan Ramaswami, PhD†

Background: Stroke is a severe emergent cardiologic disease for death and permanent disability. Ultrasound exposure could noninvasively enhance the clot lysis of tissue plasminogen activator (tPA) with the presence of microbubbles (MBs). A variety of sonography systems are available in the market, and whether sonothrombolysis is only successful using specific devices is unknown. Methods: Three commercial ultrasound systems (Siemens, Philips, and SonoSite) were characterized first for their acoustic field, and then the thrombolysis efficiencies were evaluated using the same in vitro setup and protocol. Results: Despite different acoustic fields and pressure waveforms at the focus, similar sonothrombolysis abilities with tPA and MBs were found, and the presence of temporal bone will worsen the performance slightly (by about 10% in the clot lysis). Conclusions: Therefore, it suggests that satisfactory sonothrombolysis in vivo or in clinics could be achieved using a variety of commercial ultrasound devices if the acoustic output is sufficiently strong. Key Words: Sonothrombolysis—tissue plasminogen activator—microbubble—acoustic field characterization—temporal bone.

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Introduction

Stroke is the rapid loss of brain function because of disturbance in the blood supply to the brain and can cause permanent neurologic damage, such as inability to move one or both sides of limbs, to understand or formulate speech, or to see 1 side of the visual field, and death. It is the second leading cause of death and the leading cause of permanent disability worldwide. With the high prevalence of stroke risk factors, such as high blood pressure, diabetes, and smoking, in the rapidly aging population, the burden of stroke will increase dramatically. World Health Organization reported that stroke death has increased over the years from 9.7% in 2004% to 10.8% in 2008. Strokes can be classified into 2 major categories: ischemic and hemorrhagic. Ischemic strokes are caused by interruption of the blood supply and account for 87% of all strokes. Middle cerebral artery (MCA) occlusion is the most common among stroke patients because MCA is the longest blood supplying artery to the brain but smaller in size.

Sonothrombolysis is considered an emerging modality for the treatment of acute ischemic stroke. Transcranial Doppler (TCD) or transcranial color-coded duplex (TCCD) ultrasonography that is aimed at (residual) obstructive intracranial blood flow could improve the delivery and penetration of tissue plasminogen activator (tPA) or alteplase inside the clot by mechanical pressure waves and had significantly higher rates of recanalization than patients treated with tPA alone. In a cohort study, it is found that 233 patients treated with sonothrombolysis were less likely to be dead or disabled at 3 months with no significant difference in mortality and cerebral hemorrhage. Administration of gaseous microbubbles (MBs), initially developed as ultrasound contrast agents in the cardiology diagnosis, can further enhance the effect of sonothrombolysis with a decrease in symptomatic intracranial hemorrhagic complications. Even in the absence of

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alteplase, the ultrasound energy with or without MBs could increase intrinsic fibrinolysis.\textsuperscript{8,9} However, there is an ongoing debate about the efficacy, safety, technical aspects of ultrasound administration and the possible potentiating effect of MBs.\textsuperscript{10}

Ultrasound imaging is a widely used diagnostic modality in medicine since the 1980s especially in gynecology and obstetrics because of its technical advantages, such as nonionization, low cost, mobility/portability, and high frame rate for real-time monitoring. In the market, plenty of ultrasound systems and imaging probes are available. Although certain criteria, such as acoustic intensity, acoustic power, and maximum thermal and mechanical indexes, must be met to obtain approval from the United States Food and Drug Administration for clinical use, there are significant variations in their specifications (ie, pulse duration and profile). 1.8-MHz commercial diagnostic ultrasound showed only pulsed wave Doppler-accelerated tPA-mediated thrombolysis significantly.\textsuperscript{11} Whether other sonography systems in diverse modes developed in these years could achieve satisfactory thrombolysis efficiency at the low frequency for transcranial ultrasound propagation (usually 1-2 MHz) is unknown. If so, sonothrombolysis would have a wide application owing to the facility available in most hospitals. In this study, 3 popular ultrasound systems (Siemens, Philips, and Sonosite) were compared for their acoustic fields and clot lysis abilities using the same in vitro setup and protocol.

**Materials and Methods**

**Acoustical Field Characterization**

The acoustic fields of 3 commercially available ultrasound diagnostic systems (Sequoia; Siemens, Mountain View, CA; iU22; Philips, Briarcliff, NY; MicroMaxx; Sonosite, Bothell, WA) were characterized by an acoustic measurement system (AMS; Sonora Medical Systems, Longmont, CO) with an acoustic measurement tank, a 3D translational stage with a minimum step size of 5 μm, precision ball screws to ensure both repeatability and the highest resolution, a polyvinylidene fluoride membrane hydrophone (804; Sonora Medical Systems) with a flat and stable response at 1-20 MHz, and a control program in accordance with International Electrotechnical Commission Standard 60601-2-37, “Requirements for the Declaration of the Acoustic Output of Medical Diagnostic Ultrasonic Equipment”. The polyvinylidene fluoride hydrophone was connected to a digital oscilloscope (TDS3012C; Tektronix, Beaverton, OR) at a sampling rate up to 1.25 GS/s, and the digitized waveforms were then transferred to the control personal computer for data analysis. The pulse intensity integral, \( PII \), is determined as

\[
PII = \int_{t_1}^{t_2} I(t) dt = \frac{1}{2} p_0 c_0 \int_{t_1}^{t_2} p^2(t) dt
\]

where \( I(t) \) is the acoustic intensity, \( p(t) \) is the acoustic pressure, \( p_0 \) and \( c_0 \) are the density and speed of sound in water, and \( t_1 \) and \( t_2 \) are the beginning and end of the pulse, respectively. The specifications of the ultrasound systems are listed in Table 1, and the corresponding acoustic fields were mapped in 3D space, from which the characteristics of the pulse at the focus and \(-6\) dB beam size were calculated.

**Clot Preparation**

To prepare the blood clot, 25 mL of 100 mM calcium chloride (Sigma-Aldrich, St. Louis, MO) was mixed with 1 mL of anticoagulated bovine blood (Quad Five, Ryegate, MT). After 1 minute, 1.25 mL of the mixture was injected into a silicone tube (3 mm inner diameter; PHYWE Systems GmbH, Göttingen, Germany), whose dimensions were chosen to match the size of adult human MCA. Precautions were taken to avoid the introduction of any air

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<th>Table 1. A comparison of ultrasound settings in the sonothrombolysis experiment and the pressure waveforms at the focus measured in the acoustic field characterization</th>
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<td><strong>Ultrasound system</strong></td>
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<td>( IS_{PAA.0} ) (W/cm²)</td>
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<td>(-6) dB beam size (mm × mm)</td>
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Abbreviations: \( f_0 \), central frequency; \( F \), focal length; \( IS_{PAA.0} \), spatial peak pulse average acoustic intensity; MBD, microbubble destruction; MI, mechanical index; \( Pr.0 \), rarefractional pressure; PW, pulsed wave.
bubble in the mixture. The silicone tube was then stored in an incubator (Symphony; VWR, Radnor, PA) at 37°C for 2 hours for a stable clot formation.

**Thrombolysis**

Thrombolytic capability of different ultrasound systems was evaluated in the same laboratory using our established in vitro protocol and setup (Fig 1). Cerebral artery structure was simulated using silicon tubes with an inner diameter of ~3 mm, where distilled and deionized water seeded with 20 μL tPA was circulated by a pulsatile blood pump (Harvard Apparatus, Holliston, MA) in a bolus way. Three milliliters of MRX-815 (ImaRx Therapeutics, Tucson, AZ) with a mean diameter of 1.2 μm and similar shell and gas formulation as Definity was diluted with .09% degassed saline up to 20 mL and injected into the closed loop at a flow rate of 400 μL/minute by an infusion syringe pump (KDS100; KD Scientific, Holliston, MA). A piece of blood clot (~20 mm in length) was inserted into the silicone tube and then moved to a position where a plastic stopper with several small through holes (~5 mm in diameter) is to simulate the occlusion. The water temperature in the testing tank was kept at 37°C by an immersible water circulator (7312; Polyscience, Niles, IL). Ultrasound probe was immersed in a Lucite testing tank (L × W × H = 80 × 60 × 30 cm³), and the blood clot was identified in the diagnostic ultrasound images (B-mode and power Doppler) and then aligned to the focus. After 1-hour treatment, the blood clot sample was taken out, washed with phosphate buffered saline solution thrice, and dried with Whatman filter paper. Thrombolysis efficiency was defined as the percentage of weight loss of blood clot with respect to its initial weight measured by a digital analytical balance (ML54; Mettler Toledo, Columbus, OH). In addition, a piece of temporal bone from human cadaver skull after obtaining the approval from institute review board was attached to the ultrasound probe to simulate the transcranial effect.

**Statistical Analysis**

At each testing condition, the sample size was at least 5. Analysis of variance was performed in SPSS Statistics (IBM Software, Somers, NY) to determine the statistical difference between the groups that was fixed at P < .05.
Results

Acoustic Field and Waveform

Using the automatic acoustic field mapping system, each ultrasound probe was characterized at its maximum power output or exclusive mode for sonothrombolysis as suggested by the manufacturers. The distribution of PIIs along the beam axes and the acoustic pressure waveforms at the foci are shown in Figure 2, and the quantitative values are listed in Table 1. It is found that there are statistical differences among these systems \((P < .05)\). For example, acoustic pulse generated from P17 probe of SonoSite MicroMaxx has the largest amplitude \((p^1 = \sim 6.8 \text{ MPa}, p^2 = 2.5 \text{ MPa})\) with noticeable formation of the shock front but the shortest duration \((1.58 \mu\text{s})\). So it has the maximum pulse energy \((4.00 \times 10^{-2} \mu\text{J})\) and acoustic intensity \((720 \text{ W/cm}^2)\). In contrast, pulses of Philips and Siemens systems are much longer \((5.63 \mu\text{s} \text{ and } 4.12 \mu\text{s}, \text{ respectively})\) and less noteworthy in the waveform distortion. Furthermore, SonoSite has the smallest \(-6 \text{ dB} \text{ beam size} \((2.3 \times 4.9 \text{ mm})\), which is because of its high frequency.

Sonothrombolysis

The blood clot after the thrombolysis experiment became smaller in size and paler in color (Fig 3), which illustrates the removal of red blood cells from the fibrin network, and sonothrombolysis with microbubbles could further reduce the size and weight of blood clot. Corresponding changes were also observed in the zoomed sonography (Fig 4). Thrombolysis efficiencies of different systems were evaluated using our established protocols and in vitro setup (Fig 5). Placing the blood clot samples into the MCA mimicking system with pulsatile flow alone as the control caused the percentage of weight loss in the range from 13.9\% to 23.4\% both without and with the temporal bone. Introduction of tPA could increase the thrombolytic ability \((28.7\%-36.3\%)\) with significant difference \((P < .05)\). If there is no temporal bone in front of the ultrasound probe, SonoSite had the highest sonothrombolysis \((46.6 \pm 3.6\%)\), which may be because of its high acoustic intensity and pulse energy, while those values of Siemens and Philips systems were a little higher than using tPA but without statistical difference \((P > .05)\). However, the average percentage of weight loss of the blood clot would be more than 50\% when MRX-815 was infused with the bolus injection of tPA in the ultrasound exposure. Siemens system working at the MB destruction mode achieved the maximum increase \((57.2 \pm 5.7\%)\) although the details of this mode are not known because of the technical nondisclosure. When the temporal bone was present, the sonothrombolysis with tPA were a little higher \((P > .05)\), which may be because of the larger beam width after the acoustic wave propagation through the skull. However, the corresponding ability with MBs and tPA together decreased (ie, to 47.5 \pm 3.3\% for Siemens system), but without statistical significance in comparison with tests using tPA under sonication only.

Discussion and Conclusions

In this study, 3 ultrasound imaging systems were characterized, and then their abilities of thrombolysis were evaluated using the same setup and protocol. It is found that despite the significant differences in the acoustic field characteristics (ie, pulse profile and beam width) they can achieve comparable and satisfactory outcomes. With the presence of a human temporal bone, thrombolysis efficiencies with tPA were similar to those without it whereas the corresponding values of using tPA and MBs together decreased slightly, which is because of the effect of temporal bone (ie, attenuation and diffraction) to the ultrasound burst.\(^\text{12}\) Our results suggest that sonothrombolysis technology is not dependent on a certain device, and many available ultrasound systems may have satisfactory performance.
Numerous clinical trials are ongoing to evaluate sonothrombolysis at different stages. In human stroke, the CLOTBUST (combined lysis of thrombus in brain ischemia with transcranial ultrasound and systemic tPA; design of a randomized trial of ultrasound-enhanced thrombolysis for acute ischemic stroke) phase II trial showed that the combination of alteplase and 2 hours of continuous TCD increased recanalization rates and better functional outcomes compared with alteplase alone. A multicenter international Transcranial Ultrasound in Clinical Sonothrombolysis Trial was also encouraging and illustrated that MBs can be safely administered with alteplase and TCD. If approved finally, sonothrombolysis can be applied using a variety of sonographic approaches besides TCD and TCCD, such as MB destruction.

However, mechanisms of sonothrombolysis are not clearly understood. Bubble cavitation could be produced close to the blood clot under ultrasound exposure, and different microstreaming patterns can be formed in the vicinity of the oscillation bubbles. Consequent shear stress and stretch/compression would accelerate the diffusion of thrombolytic drug into the fibrin matrix. The asymmetric collapse of inertial cavitation bubble can lead to intense localized stress forces and the production of a high-speed microjet toward the surface of the blood clot to alter the structure of fibrin networks (ie, thrombus disintegration) and to increase the permeability. Introduction of MBs can lower the ultrasound energy threshold needed to induce acoustic cavitation. MBs can readily penetrate into fibrin clots to form tunnels with a diameter of 9-35 μm according to the acoustic radiation force. Because the threshold of bubble cavitation and acoustic attenuation of skull decrease with the driving frequency, enhanced sonothrombolysis at the low frequency is expected. However, Thrombolysis in Brain Ischemia Study unfortunately resulted in a higher rate of symptomatic intracranial hemorrhage and occurrence of cerebral bleeding on the opposite side of the brain infarction at driving frequency of 20-300 kHz, which may be because of the established standing wave inside the skull. The presence of temporal bone did not weaken the thrombolysis significantly at much high frequency as shown in this study, but the availability of the penetration window in patients is a big concern in the clinical application of sonothrombolysis. Soft brain tissue will further attenuate the acoustic wave but not in a high degree as the skull bone. Presence of the temperature bone in this study reduced the sonothrombolysis efficiency in the range of 4.5%-16.4%. In addition, optimal acoustic parameters and profile for sonothrombolysis need further investigation based on the understanding of the mechanism. Randomized controlled clinical trials of 2-MHz TCD and 1.3-2 MHz and 1.8-MHz TCCD combined with tPA had statistically significant higher rates of recanalization than patients treated with tPA alone but did not lead to an increase in symptomatic intracranial hemorrhagic complications. Therefore, it is reasonable to expect safety of the systems used in this study (1.3 MHz for Siemens Sequoia, 1.6 MHz for Philips iU22, and 2.5 MHz for SonoSite MicroMaxx) because the cavitation threshold is inversely proportional to the ultrasound driving frequency.

In summary, these 3 commercial ultrasound systems tested in this study have different characteristics of acoustic field and pressure waveform at the focus but similar sonothrombolysis abilities with tPA and MBs, and...
presence of temporal bone will not worsen the performance considerably. Therefore, sonothrombolysis in vivo or in clinics may be produced using a variety of ultrasound devices. Additional work is needed to optimize the ultrasound parameters and probe configuration for enhanced clot lysis, recanalization, and reduced symptomatic intracranial hemorrhage in vivo.

References