Review Article

Review of Protocols Used in Ultrasound Thrombolysis

Nicos Papadopoulos, PhD,* Panayiotis A. Kyriacou, PhD,* and Christakis Damianou, PhD†

Objectives: This paper focuses on the review of protocols used in thrombolysis studies with ultrasound. *Materials and methods:* Data from peer-review articles were acquired. *Results:* The protocols of several published reports are summarized in 3 tables (in vitro, in vivo, and clinical), providing detailed information concerning clot model, thrombolytic drug, treatment mode, sonication param-eters, evaluation method, thrombolysis outcome, side effects, and conclusions. *Conclusions:* The aim of this review was to give an overview of the different pro-tocols used so far in the field of sonothrombolysis and investigate the impact ofseveral aspects involved on sonothrombolysis outcome.
Key Words: Stroke—thrombus—ultrasound—MRI—clot.

Introduction

One of the early investigators who used ultrasound (US) energy to accelerate the fibrinolytic activity of thrombolytic drugs in vitro was Lauer.[1] Later, more in vitro studies have shown that US applications improved thrombolysis induced by thrombolytic agents (sonothrombolysis). The main goal in these in vitro studies was to deduce the optimum ultrasonic parameters to enhance sonothrombolysis (mostly frequency and intensity).[2] Another major goal was to test the best thrombolytic drug that enhances sonothrombolysis.[3,4] The knowledge on sonothrombolysis gained in the in vitro studies was translated at a preclinical level by performing experiments in animals. Because in the in vitro experiments no side effects can be extracted, experimentation with animals was imperative. Still the main goal in the animal experiments was to extract the optimum ultrasonic parameters that maximize clot removal.[5] Additionally, different clot animal models were used which could test the various derived ultrasonic protocols.[6,7] Progressively, US bubbles were employed which can possibly enhance the efficacy of sonothrombolysis.[5] When sufficient data were collected this research was translated into clinical trials.[8-10] The main goal in the clinical trials was to establish the safety and efficacy of sonothrombolysis. As was evident from these studies, the efficacy of this method was not very encouraging, therefore its deployment was not that impressive compared with the preclinical studies. Additionally, some side effects reported delayed the full deployment of this method.

This review is divided into 3 categories (in vitro, in vivo, and clinical) and provides a comprehensive compilation of protocols used during sonothrombolysis studies with or without thrombolytic drugs and/or microbubbles (MBs) since 1992. The aim of the review is to provide information regarding (1) the clot model used (human or animal for the in vitro studies and type of occlusion for the animal and clinical studies), (2) the US technique applied such as external or internal (catheter based) and focused or unfocused, (3) the use of flow system (only

From the *Research Centre for Biomedical Engineering, City, University of London, UK; and †Electrical Engineering Department, Cyprus University of Technology, Cyprus.

in vitro), (4) the temperature (only in vitro), (5) the type and concentration of thrombolytic drug used, (6) the treatment mode (US alone, drug alone, US + drug and US + drug + MBs), (7) the sonication parameters applied, such as frequency, intensity or acoustic power or negative pressure, pulse repetition frequency (PRF), duty factor (DF), and treatment time, (8) the evaluation method used to estimate study's outcome, (9) the effect of treatment on clot lysis, and (10) the main conclusions derived.

There is an absence in standardization about the necessary information collected from each study due to different methods/measuring units used by the investigators. For example, the output of US transducer is specified in different units (intensity, acoustic power, negative pressure, etc.), and the treatment's outcome is quantified by different evaluation methods such volume reduction, fibrin degradation products, lytic rate, recanalization rate, etc. Furthermore, in some cases experimental parameters like temperature, PRF and DF are not specified. This lack of standardization makes the comparison among various studies impossible. Additionally, although some impressive results were reported in the in vitro and in the animal studies, the outcomes of the clinical results were not that impressive. This could be attributed to the fact that in some studies, thermal effects were possibly reached, causing acceleration of sonothrombolysis, which, however, eventually produced severe side effects.

Materials and Methods

Published reports on sonothrombolysis that are available in PubMed (www.ncbi.nlm.nih.gov/pubmed) were collected. Information in several aspects of the protocols used in the studies examined were also extracted. In animal studies as well as in clinical trials, the following information was needed: clot model, thrombolytic drug and concentration, treatment mode, MBs administration, frequency, intensity or acoustic power or negative pressure, PRF, DF, treatment time, evaluation method, treatment's outcome, side effects, and main conclusions. In the in vitro compilation, the additional information needed was temperature.

Results

Table 1 lists the in vitro studies, Table 2 lists the in vivo studies, and Table 3 lists the clinical studies. The 3 tables include a comprehensive summary of all the issues involved in sonothrombolysis. These main issues are (1) the clot model used (human, animal, or in vitro), (2) type of occlusion (for animal and clinical models), (3) the coupling technique used (external or internal), (4) US modality (focused or unfocused), (5) the use of flow system (only for the in vitro studies), (6) indication of temperature (only for the in vitro studies), (7) the type of thrombolytic drug used, (8) concentration of thrombolytic drug used, (9) treatment mode (US alone, drug alone, US + drug and US + drug + MBs), (10) the applied frequency, (11) the applied intensity or acoustic power or negative pressure, (12) the applied PRF, (13) the applied DF, (14) the treatment time, (15) the evaluation method used to estimate the efficacy of sonothrombolysis, (16) the effect of treatment on clot lysis, and (17) the main conclusions derived.

In the in vitro studies the most common clot model used was the human model (e.g., References 1-3, 12, and 13). In some cases the porcine model was used,[4,48,54,56-58] the rabbit model,[46,53] and the bovine.[45,49] The intensity used ranged from .5 W/cm^2 to 193 W/cm^2. The frequency used varied from 20 KHz to 2 MHz, whereas in most experiments the frequency used was about 1 MHz. The most typical thrombolytic drug used was the recombinant tissue plasminogen activator (rt-PA). In a few studies the urokinase (UK) was used.[21,25,32,36] The concentration of the rt-PA varied from .1 to 100 μg/mL. In most of the studies the drug concentration is specified as μg/mL, and in some studies the IU/mL is specified. The treatment time used varied from .5 minutes to 720 minutes, whereas the majority of the studies used treatment time between 30 and 60 minutes. We have observed that in a few studies the clot temperature was not specified (e.g., References 36, 41, and 45). Based on 1 study,[34] it is apparent that temperature plays an important role in sonothrombolysis and should be specified in all in vitro studies.

In the animal studies the most common clot models used were the rabbit model[5,6,48,64-74,78-80] and the rat model.[1,7,63,75] In the popular rabbit model the most commonly used artery was the femoral, followed by the middle cerebral artery (MCA) and the carotid. The frequency used varied from 20 kHz to 5.7 MHz, whereas in most experiments the frequency used was about 1 MHz. The most typical thrombolytic drug used was the rt-PA. In a few studies the streptokinase was used.[66,70,80] The concentration of the drug varied from .8 to 10 μg/mL. Clearly the doses used in animals were much lower than those used in the in vitro models. Most of the studies have evaluated the effect of US alone, thrombolytic drug alone, or the synergy of the 2 (US and drug). In most of the studies the intensity is specified, and in some studies the pressure is specified. The treatment time used varied from 2 minutes to 120 minutes, whereas the majority of the studies used treatment time of 60 minutes. Compared with the in vitro studies, in the animal studies, the additional parameter used was the inclusion of MBs. Several studies[71-73,78-80] have shown that MBs may enhance the sonothrombolysis efficiency.

In all the human trials evaluated the clot model used was the MCA. The frequency used varied from 300 kHz to 4 MHz. In all the studies the thrombolytic drug used was the rt-PA. The concentration of the drug was .9 μg/mL, which seems to be the safe dose used in humans.

Table 1. *In vitro protocols used in sonothrombolysis*

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human	rt-PA (3000 IU/mL)	37		Yes							50	Measured volume reduction	33%	[1]
					Yes		1 MHz	1.75 W/cm^2 I_{SATA}	Intermittent	50	50		50%	
				Yes							200		62%	
					Yes		1 MHz	1.75 W/cm^2 I_{SATA}	Intermittent	50	200		91% US combined with rt-PA caused a significant enhancement of thrombolysis compared with rt-PA alone.	
Human	rt-PA (1 µg/mL)	37		Yes							60	Measured volume reduction	8%	[2]
					Yes		27 kHz	1 W/cm^2	70	10	60		17%	
					Yes		40 kHz	1 W/cm2	70	10	60		20%	
					Yes		100 kHz	1 W/cm2	70	10	60		15% US in the range of 27 to 100 kHz is effective in accelerating fibrinolysis at intensities and pulsing conditions that minimize the probability of heating and cavitation.	
Human	rt-PA (3000 IU/mL)	36		Yes							60	Measured volume reduction	22.7%	[11]
					Yes		2 MHz	1.2 W/cm^2	cw	100	60		49% (Traveling)	
					Yes		2 MHz	1.2 W/cm^2	cw	100	60		34.8% (Standing)	
					Yes		2 MHz	1.2 W/cm^2	1, 10, 100, 1000	50	60		46.4%, 39.8%, 44%, 34% Intermittent application of a 2-MHz high-frequency US using a traveling wave field would be the most potent application for lysing blood clots. No effect of PRF on clot lysis.	
Human	rt-PA (3000 IU/mL)	37	Yes				1.95 MHz	1.2 W/cm^2 I_{SATA}	1	50	60	Measured volume reduction	25.3%	[3]
				Yes							60		19.9%	
					Yes		1.95 MHz	1.75 W/cm^2 I_{SATA}	1	50	60		35.2% US enhances thrombolysis by affecting the distribution of rt-PA within the clot.	
Human	rt-PA (3.15 µg/mL)	37		Yes							30	Measured lytic rate	.5 µm/min	[12]
					Yes		120 kHz	.35 MPa	1667	80	30		3.4 µm/min US treatment + rt-PA significantly enhanced the mean lytic rate (580% change), compared with rt-PA treatment alone.	
Human	rt-PA (3.15 µg/mL)	37		Yes							30	Measured lytic rate	7 µm/min	[13]
					Yes		120 kHz	.35 MPa	1667	10, 20, 50, 80	30		15, 25, 50, 65 µm/min The lytic efficacy of clots exposed to rt-PA and US increases with increasing DF.	
Porcine	rt-PA (107 µg/mL)	37		Yes							30	Measured volume reduction	12%	[4]
					Yes		120 kHz	.35 MPa	1.7 k	80	30		19.1%	
					Yes		1 MHz	.35 MPa	1.7 k	100	30		25%	
					Yes		1 MHz	.35 MPa	1.7 k	10	30		22% Both 120-kHz and 1-MHz pulsed and c.w. US enhanced rt-PA thrombolysis in a porcine whole blood clot model.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human	rt-PA (10 µg/mL)	37		Yes							30	Measured volume reduction	16%	[14]
					Yes		120 kHz	.35 MPa	1667	50	30		31% 120-kHz US substantially increases the lytic efficacy of rt-PA for almost all rt-PA concentrations.	
Human	rt-PA (3.15 µg/mL)	37		Yes							30	Measured volume reduction	31%	[15]
					Yes		120 kHz	.35 MPa	1667	50	30		71%	
				Yes		Yes (t-ELIP)					30		48%	
					Yes	Yes (t-ELIP)	120 kHz	.35 MPa	1667	50	30		89% The addition of 120 kHz US with t-ELIP substantially increases the rate of lysis compared with either tPA or t-ELIP alone.	
Human	rt-PA (.5-3.15 µg/mL)	37		Yes							30	Measured volume reduction	38%-44%	[16]
					Yes		120 kHz	.18 MPa	1667	50	30		50%-70%	
					Yes		2 MHz	.47 MPa	10.5 k	13	30		52%-58% Combination treatment with rt-PA is more effective than rt-PA alone in human whole blood clots.	
Fibrin gel	rt-PA (.1 µg/mL)	25			Yes		1 MHz	2 W/cm^2	100	50		Measured binding ratios	US exposure accelerates rt-PA binding, alters binding affinity, and increases maximum binding to polymerized fibrin	[17]
Fibrin gel			Yes				1 MHz	4 W/cm^2	c.w.	100	15	Measured fiber density Measured fiber diameter	>65% <27% US exposure causes reversible disaggregation of uncross-linked fibrin fibers into smaller fibers, an effect that may alter flow resistance and create additional binding sites for fibrinolytic components, improving fibrinolytic efficacy.	[18]
Human	rt-PA (.1 µg/mL)	37		Yes							240	Measured uptake rate	8.2%	[19]
					Yes		1 MHz	4 W/cm^2	c.w.	100	240		15.5% Exposure to US increases uptake of rt-PA into clots and also results in deeper penetration.	
Human	rt-PA (2 µg/mL)			Yes							60	Measured fibrin degradation product, D-dimer (FDP-DD)	957 ng/mL	[20]
					Yes		300 kHz	.07 W/cm2	c.w.	100	60		1669 ng/mL	
					Yes		1 MHz	.4 W/cm^2	c.w.	100	60		1727 ng/mL The combination of drug and US leads to degradation of fibrin, allowing a quantitative measurement of the enhancement of clot lysis. A high correlation was observed between the FDP-DD produced with the rate of decrease in clot weight.	
Human flow system	UK (400 U/mL)	37		Yes							60	Measured volume reduction	18.7%	[21]
					Yes		1 MHz	2.5 W/cm^2	c.w.	100	60		52.5% c.w. US at 1 MHz and an intensity of 2.5 W/cm2 accelerates urokinase-induced thrombolysis and reperfusion.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human	SK (5000 U/mL)	37		Yes							65	Measured reperfusion rate	100%	22
					Yes		170 kHz	.5 W/cm^2 (I_{SATA})	c.w.	100	34		100%	
				Yes							39		100%	
					Yes		1 MHz	1 W/cm^2 (I_{SATA})	c.w.	100	20		100% US exposure of a type and intensity that may be transmitted transthoracically accelerates the thrombolytic process (for both frequencies used).	
Human HIFU	rt-PA (10 µg/mL)	37	Yes				1 MHz	60 W	1	10	30	Measured volume reduction	33%	23
				Yes							30		53.1%	
					Yes		1 MHz	60 W	1	10	30		63.2% The rate of tPA-mediated thrombolysis can be enhanced by using pulsed HIFU exposure.	
Human	rt-PA (1.0 µg/mL)	37		Yes							60	Measured volume reduction	12.8%	24
					Yes		1 MHz	1, 2, 4, 8 W/cm^2	c.w.	100	60		18%, 19.3%, 22.8%, 58.7% US at 1 MHz potentiates enzymatic fibrinolysis by a nonthermal mechanism. Thrombolysis efficiency increases with intensity.	
Human	UK (200, 2000, 5000 µg/mL)	Room	Yes				1 MHz	2.2 W/cm^2	n/s	n/s	30	Measured volume reduction	18%	25
				Yes							30		19%, 44%, 50%	
					Yes		1 MHz	2.2 W/cm^2	n/s	n/s	30		41%, 55%, 61%	
	SK (50, 250, 2000 µg/mL)		Yes				1 MHz	2.2 W/cm^2	n/s	n/s	30		26%	
				Yes							30		37%, 50%, 62%	
					Yes		1 MHz	2.2 W/cm^2	n/s	n/s	30		45%, 61%, 73% The use of external US has the potential to increase both efficacy and rate of thrombolysis. Thrombolysis efficiency increases with drug dose. Thrombolytic activity of SK more effective than UK.	
Human	rt-PA (1.0 µg/mL)	37		Yes							60	Measured volume reduction	23%	26
					Yes		2.2 MHz	.5, 1, 2, 4, 8 W/cm^2	n/s	n/s	60		31%, 40%, 48%, 69%, 88% US accelerates enzymatic fibrinolysis by increasing transport of reactants through a cavitation-related mechanism. Thrombolysis efficiency increases with intensity.	
Human	rt-PA (66.7 µg/mL)	37		Yes							25	Measured volume reduction	7.24%	27
					Yes		.5 MHz	8 W/cm^2	c.w.	100	25		26.7% US waves accelerate rt-PA-induced thrombolysis and reperfusion.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human	rt-PA (1 µg/mL)	37		Yes							60, 120	Measured volume reduction	13%, 37%	28
					Yes		40 kHz	.25 W/cm^2	c.w.	100	60, 120		39%, 93%	
					Yes		40 kHz	.75, 1, 1.5 W/cm^2	c.w.	100	60		58%, 75%, 77% 40-kHz US significantly accelerates enzymatic fibrinolysis with excellent tissue penetration and minimal heating. Thrombolysis efficiency increases with intensity.	
Human	rt-PA (3000 U/mL)	37		Yes							60	Measured volume reduction	23.8%	29
					Yes		1 MHz	1.2 W/cm^2	c.w.	100	60		34.8 (Standing)	
				Yes							60		11.3%	
					Yes		2 MHz	1.2 W/cm^2	c.w.	100	60		24.5% (Traveling) Traveling US waves enhanced thrombolysis (116.8%), which is significantly more than standing US waves did (46%).	
Human	rt-PA (3 µg/mL)	37	Yes				20 kHz	.35 W/cm^2	c.w.	100	10	Measured volume reduction	41.8%	30
				Yes							20		49.1%	
					Yes		20 kHz	.35 W/cm^2	c.w.	100	10		65.8% The use of low-frequency US alone has the potential to induce thrombolysis. Combination of US with rt-PA is superior to either treatment alone.	
Human	rt-PA (3.15 µg/mL)	37		Yes							30	Measured volume reduction	15.6%	31
				Yes + Epf.							30		28%	
					Yes		120 kHz	.18 MPa	1667	80	30		44.4%	
					Yes + Epf.		120 kHz	.18 MPa	1667	80	30		30.3% Although the addition of eptifibatide enhances the lytic efficacy of rt-PA alone, the efficacy of US and rt-PA is greater than that of combined US, rt-PA, and eptifibatide exposure.	
Human	UK (2 mg/mL)	37		Yes							720	Measured volume reduction	43%	32
					Yes		211.5 kHz	.25 W/cm^2	c.w.	100	720		61% Low-frequency US transmits well through human temporal bone and enhances thrombolysis.	
Human through skull	rt-PA (100 µg/mL)	37	Yes				33.3 kHz	.5 W/cm^2	n/s	n/s	60, 180	Measured volume reduction	39.96%, 44.09%	33
			Yes				71.4 kHz	3.4 W/cm^2	n/s	n/s	60, 180		32.24%, 35.17%	
				Yes							60, 180		46.55%, 56.27%	
					Yes		33.3 kHz	.5 W/cm^2	n/s	n/s	60, 180		51.04%, 67.89%	
					Yes		71.4 kHz	3.4 W/cm^2	n/s	n/s	60, 180		46.23%, 60.47% Transcranial application of US can shorten the recanalization time of intracerebral vessel occlusion by increasing rt-PA-mediated thrombolysis. Thrombolysis efficiency increases with time.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human	rt-PA (3.15 µg/mL)	33, 37	Yes				120 kHz	3.2 W/cm^2	1667	80	30	Measured volume reduction	7.5%, 7.2%	34
				Yes							30		8.6%, 12.4%	
					Yes		120 kHz	3.2 W/cm^2	1667	80	30		21.2%, 22.7% The efficacy of US enhanced thrombolysis decreases at temperatures below the body baseline temperature of 37°C.	
Human through temporal bone	rt-PA (10 µg/mL)	37	Yes				1.8 MHz	1.6 MI	n/s	n/s	60	Measured volume reduction	41% 70.8%	35
				Yes							60		78.7%	
					Yes		1.8 MHz	1.6 MI	n/s	n/s	60		Diagnostic transcranial US with rt-PA, enhances thrombolysis.	
Human through skull HIFU flow system		n/s	Yes				220 kHz	111 W/cm^2	2.5	50	.5	Measured volume reduction	76.1% (Flow)	36
		n/s	Yes				220 kHz	111 W/cm^2	2.5	50	.5		29.9% (No flow) Trans-skull HIFU for immediate clot lysis without the need of further drugs and disregarding individual skull bone characteristics is feasible.	
Human	UK (1200 IU)	37		Yes							60	Measured volume reduction after incubation	40.6%	37
					Yes		48 kHz	5-6 kPa	n/s	n/s	60		59.2%	
				Yes							60, 120		8.9%, 46.7%	
					Yes		225 kHz	30 mW/cm^2	n/s	n/s	60, 120		37.3%, 61.1% US energy enhanced fibrinolysis with UK, especially in the early phase of lysis. Thrombolysis efficiency increases with time and decreases with frequency.	
Bovine through skull flow system	rt-PA (100 µg/mL)	37		Yes							29.3	Measured recanalization rate	100%	38
		37			Yes		1 MHz	.35 W/cm^2(I_{SPTP})	16 k	41.6	17.1		100%	
					Yes		185 kHz	1.27 W/cm2 (I_{SPTP})	c.w.	100	14.1		100% Transcranial application of low frequency, c.w. US may accelerate reperfusion and shorten the recanalization time.	
Bovine through skull flow system	rt-PA (100 µg/mL)	37		Yes							30	Measured recanalization rate	30%	39
					Yes		1 MHz	.35 W/cm^2 (I_{SPTP})	16,000	41.6	30		90%-100% Transcranial application of 1 MHz US may accelerate reperfusion and recanalization rate of occluded intracerebral vessels.	
Human through skull HIFU flow system		24	Yes				220 kHz	13.7, 27.4, 54.8, 136.8 W/cm^2 (I_{SPTA})	.5, 5, 50, 500	5, 10, 20, 50	.5	Measured volume reduction	10.3%-27.2% 17.1%-42.9% 30%-59.6% 48.7%-59.2% Using transcranial HIFU, significant thrombolysis can be achieved within seconds and without the use of lytic drugs. Longer DF in combination with longer p.w. seems to have the highest potential to optimize clot lysis efficacy.	40

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human FUS		n/s	Yes				550 kHz	200 W	3.7	10	5	Measured volume reduction	80%	41
			Yes				535 and 565 kHz	110 W	3.7	10	5		80% The power needed to achieve 80% of thrombolysis with a monofrequency excitation is reduced by half with a bifrequency excitation.	
Human	rt-PA (60 kU/mL)	37		Yes							60	Measured volume reduction	36.7%	42
					Yes		2 MHz TCCD	.179 W/cm^2	n/s	n/s	60		40.8%	
					Yes		2 MHz TCD	.457 W/cm^2	n/s	n/s	60		40.4% Although clot lysis rate after 1-h treatment with rt-PA alone was significant, a slight increase of weight loss was detected under the application of US + drug.	
Human HIFU		37	Yes				230 kHz	1000 W	1000	10	.5	Measured volume reduction	82% After sonication, the clot was nearly completely lysed.	43
Human through skull HIFU flow system		37	Yes				220 kHz	29.71-193.24 W/cm^2 (I_{SPTA})	2.5	50	.5	Measured volume reduction	4.55%-74.83% Transcranial sonothrombolysis could be achieved within seconds in the absence of rt-PA and without producing relevant clot fragmentation, using acoustic output powers of <400 W.	44
Bovine		n/s	Yes				500 kHz	I_{SPTA} > 35 W/cm^2	200	4	4	Measured volume reduction	91% External HIFU thrombolysis for periods of ≤5 min appears to be a safe and effective method to induce thrombolysis.	45
Rabbit flow model		Room	Yes				1.51 MHz	185 W	1	.1	.33	Measured volume reduction	99.2% HIFU thrombolysis is feasible as a means of restoring partial blood flow in thrombus occluded arteries in the absence of thrombolytic agents.	46
Human	rt-PA (10 µg/mL)	37	Yes				1 MHz	4 W/cm^2 (I_{SATA})	c.w.	100	60	Measured volume reduction	6.8% at 0 atm	47
				Yes							60		21.8% at 0 atm	
					Yes		1 MHz	4 W/cm^2 (I_{SATA})	c.w.	100	60		39.3% at 0 atm US is ineffective in increasing fibrinolysis without a fibrinolytic agent present. An 80% increase in clot lysis occurs when US and agent are both present (no overpressure).	
Porcine	rt-PA (107 µg/mL)	37		Yes							30	Measured volume reduction	13%	48
					Yes		120 kHz	.15 MPa	1667	80	30		13.7% (<SC)	
					Yes		120 kHz	.24 MPa	1667	80	30		26% (SC)	
					Yes		120 kHz	.36 MPa	1667	80	30		20.7% (SC + IC) Significant enhancement of thrombolysis correlates with presence of cavitation. Stable cavitation appears to play a more important role in the enhancement of thrombolysis.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human catheter type flow system	rt-PA (300 µg/mL)	37		Yes							10	Measured volume reduction	13.6%	49
					Yes		1.7 MHz	62.46 W/cm^2 (I_{SPPA})	30	8.5	10		21.4%	
					Yes		1.7 MHz	180.02 W/cm^2 (I_{SPPA})	40	4	10		46.1% Thrombolysis efficiency increases with intensity.	
Human	rt-PA (96 µg/mL)	37	Yes			Yes	120 kHz	.32 MPa	1667	80	30	Measured volume reduction	6.3%	50
				Yes							30		13.0%	
					Yes		120 kHz	.32 MPa	1667	80	30		16.0%	
					Yes	Yes	120 kHz	.32 MPa	1667	80	30		26.2% MBs administration further increases the effect of US on rt-PA induced thrombolysis.	
Bovine	rt-PA (1000 IU/mL)	37	Yes			Yes	500 kHz	.7 W/cm^2	c.w.	100	1	Measured volume reduction	25.8%	49
				Yes							1		24.2%	
					Yes		500 kHz	.7 W/cm^2	c.w.	100	1		24.9%	
					Yes	Yes	500 kHz	.7 W/cm^2	c.w.	100	1		29.2% MBs have slightly accelerated the thrombolytic effect of rt-PA.	
Human Catheter type	rt-PA (5000 IU/mL)	37	Yes			Yes	1.7 MHz	4.9 W/cm^2 I_{SATA}	n/s	n/s	30	Measured volume reduction	.95%	51
				Yes							30		7.68%	
					Yes		1.7 MHz	4.9 W/cm^2 I_{SATA}	n/s	n/s	30		11.10%	
					Yes	Yes	1.7 MHz	4.9 W/cm^2 I_{SATA}	n/s	n/s	30		14.41% SC plays an important role in MB-enhanced US accelerated rt-PA-mediated thrombolysis.	
Human flow system	rt-PA (20 µg/mL)	37	Yes				2 MHz	455 mW/cm^2 I_{SPTA}	5000	n/s	30	Measured volume reduction	6.1%	52
			Yes			Yes		455 mW/cm^2 I_{SPTA}	5000	n/s	30		10.9%	
					Yes		2 MHz	455 mW/cm^2 I_{SPTA}	5000	n/s	30		13.1%	
					Yes	Yes	2 MHz	455 mW/cm^2 I_{SPTA}	5000	n/s	30		30.7% The application of MBs strongly accelerates lysis of clots exposed to low-intensity US with rt-PA.	
Rabbit		25	Yes			Yes (3 µm)	1 MHz	.1 W/cm^2	100	20	30	Measured volume reduction	18%	53
			Yes			Yes (1 µm)	3 MHz	2 W/cm^2	100	20	30		18% Sonothrombolysis efficacy was achieved at 20-fold lower intensity with 3 µm MBs (.1 W/cm2) than with 1 µm MBs (2.0 W/cm2).	
Porcine flow system	rt-PA (7.1 µg/mL)	37		Yes							30	Measured volume reduction	29%	54
				Yes		Yes	120 kHz	.44 MPa	c.w.	100	30		34%	
					Yes	Yes	120 kHz	.44 MPa	c.w.	100	30		83% (SC) SC nucleated by an infusion of MBs enhances rt-PA thrombolysis without apparent treatment-related damage.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Human flow system	rt-PA (3 µg/mL)	37		Yes							60	Measured clot diameter loss	6.6 µm/min	55
	rt-PA (.3 µg/mL)				Yes	Yes	1.6 MHz	600 kPa	.33	33	60		5.9 µm/min The combination of US, MB, and a low dose of rt-PA (.3 µg/mL) is as effective for thrombolysis as is a high dose of rt-PA (3 µg/mL) alone.	
Porcine flow system	rt-PA (3 µg/mL)	37		Yes							30	Measured volume reduction	25.6%	56
					Yes	Yes	1 MHz	1 MPa	.2	.002	30		55.7% The US + MB + rt-PA treatment showed dramatically higher lytic efficacy than rt-PA treatment alone.	
Porcine Flow system	rt-PA (1 µg/mL)	37	Yes			Yes	1 MHz	1.5 MPa	.34	.17	20	Measured lytic efficacy In terms of pressure change (thrombotic occlusion = 40 mm Hg)	6 mm Hg	57
					Yes	Yes	1 MHz	1 MPa	.34	.17	20		2 mm Hg Similar lytic efficacy was achieved at 1.5 MPa without rt-PA as was at 1.0 MPa with rt-PA.	
Porcine flow system			Yes			Yes	1.6 MHz	.2 (MI)	n/s	n/s	10	Measured volume reduction	54% (20 µs PD)	58
			Yes			Yes	1.6 MHz	.2 (MI)	n/s	n/s	10		33% (5 µs PD) Slightly prolonging the pulse duration (PD) on a diagnostic transducer improves the degree of sonothrombolysis that can be achieved without fibrinolytic agents at a lower MI.	
Human flow system	rt-PA (.32-3.15 µg/mL)	37.3		Yes							10	Measured lytic rate	.8%-2%/min	59
					Yes		120 kHz	.44 MPa	Inter.	62.5	30		.8%-2%/min	
					Yes	Yes	120 kHz	.44 MPa	Inter.	62.5	30		2-5%.5%/min MBs administration significantly enhanced lytic rate. Both SC and radiation force are mechanistically responsible for the process of clot lysis.	
Human flow system	rt-PA (3 µg/mL)	37		Yes							60	Measured fibrin degradation product (FDP)	51.7%	60
					Yes	Yes	1 MHz	528 mW/cm^2 I_{SPTA}	.8	40	60		53.2% (SC)	
					Yes	Yes	1 MHz	323 mW/cm^2 I_{SPTA}	.8	8	60		57.2% (SC + IC)	
					Yes	Yes	1 MHz	3 mW/cm^2 I_{SPTA}	.8	.08	60		50.9% (SC + IC)	
					Yes	Yes	1 MHz	45 mW/cm^2 I_{SPTA}	.8	.08	60		66.3% (IC) Both SC and IC, resulting from the US–MB interaction, increased the efficacy of rt-PA with respect to fibrin degradation.	

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Table 1. (*continued*)

Clot model	Drug	Temp. (°C)	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
Porcine FUS flow system	rt-PA (3.5 µg/mL)	37	Yes			Yes	1 MHz	20 W	100	10	30	Measured volume reduction	31%	61
				Yes							30		45%	
					Yes		1 MHz	20 W	100	10	30		56.2%	
					Yes	Yes	1 MHz	20 W	100	10	30		69.5% MBs administration further enhanced the beneficial effect of FUS on TNK-tPA mediated thrombolysis.	
Human flow system	rt-PA (7 µg/mL)	37		Yes							30	Measured volume reduction	29.2%	62
					Yes		.6 MHz	60 W	100	10	30		45.8%	
					Yes		1 MHz	20-60 W	100	10	30		39%-62.5%	
					Yes	Yes	1 MHz	60 W	100	10	30		87.5%	
													1 MHz FUS frequency is associated with enhanced thrombolysis compared with that of .6 MHz. An increased linear relationship between acoustic power and thrombolysis efficacy was exhibited. The combination of MBs + FUS strongly enhanced the thrombolytic efficacy of TNK-tPA.	

Abbreviations: Abib, abciximab immunobubbles; c.w., continuous wave; DF, duty factor; ELIP, echogenic liposomes; Epf., eptifibatide; Freq., frequency; FUS, focused ultrasound; HIFU, high-intensity focused ultrasound; IA, intrarterial; IV, intravenous; MBs, microbubbles; MCA, middle cerebral artery; mt-PA, monteplase tissue plasminogen activator; n/s, not specified; Nsib, nonspecific immunobubbles; PRF, pulse repetition frequency; p.w., pulsed wave; Ref., references; rt-PA, recombinant tissue plasminogen activator; sICH; symptomatic intracranial hemorrhage; SK, streptokinase; TCD, transcranial Doppler; TCCD, transcranial color-coded duplex; Temp., temperature; TNK-tPA, tenecteplase tissue plasminogen activator; UK, urokinase; US, ultrasound.

Table 2. *In vivo protocols used in sonothrombolysis*

Clot model	Drug	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
		US alone	Drug alone	US + drug										
Rat Jugular vein occlusion	rt-PA (1 mg)		Yes							50, 200	Measured volume reduction	2%0, 30%		1
				Yes		1 MHz	1.75 W/cm^2	Intermittent	50	50, 200		41%, 55%		
	rt-PA (2 mg)		Yes							50, 200		28%, 40%		
				Yes		1 MHz	1.75 W/cm^2	Intermittent	50	50, 200		45%, 50%		
												Intermittent US administered during a 200-min period showed a trend toward enhancement of rt-PA-induced fibrinolysis without causing thermal changes or inducing tissue damage. Thrombolysis efficiency increased with drug dose.		
Rat MCA stroke	rt-PA (10 mg/kg)		Yes							60	Measured relative infarct volume reduction	34%	18% ICH	63
	rt-PA (5 mg/kg)			Yes		25.6 kHz	.6 W/cm^2	n/s	20	60		51%		
	rt-PA (10 mg/kg)			Yes		25.6 kHz	.6 W/cm^2	n/s	20	60		68%	20% ICH	
												US treatment in addition to rt-PA is more effective than single rt-PA treatment in reducing infarct volume and safe with regard to bleeding. Thrombolysis efficiency increased with drug dose.		
Rabbit Embolic stroke HIFU		Yes				1.5 MHz	255 W	1	.1	.33	Measured reperfusion rate	0% (0/3)		64
		Yes				1.5 MHz	415 W	1	.1	.33		50% (2/4)		
		Yes				1.5 MHz	550 W	1	.1	.33		70% (5/7)	20% ICH	
												HIFU, as a stand-alone method, can cause effective thrombolysis and does not damage the targeted vessels.		
Rabbit MCA Stroke HIFU		Yes			Yes	1.5 MHz	88-137 W	1	.1	.33	Measured recanalization rate	78% (7/9)	22% ICH	65
		Yes			Yes	1.5 MHz	88 W	10	.1	.33		50% (1/2)		
												Droplets reduce the IC threshold and enable IC-mediated clot lysis to occur at lower power levels.		
Rabbit Femoral artery occlusion HIFU		Yes				1.51 MHz	185 W	1	.1	.33	Measured flow restoration rate	0% (0/5)		48
		Yes				1.51 MHz	215 W	1	.1	.33		50% (1/2)		
		Yes				1.51 MHz	300 W	1	.1	.33		63% (5/8)	13% ICH	
												HIFU thrombolysis is feasible as a means of restoring partial blood flow in thrombus-occluded arteries in the absence of thrombolytic agents. Increased power resulted in increased flow restoration rate.		

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Table 2. (*continued*)

Clot model	Drug	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
Rabbit Iliofemoral artery occlusion	SK (25000 U/kg)			Yes		37 kHz	160 W	91	n/s	15	Measured recanalization rate	40% (6/15)		66
				Yes		37 kHz	160 W	91	n/s	30		67% (10/15)		
				Yes		37 kHz	160 W	91	n/s	45		87% (13/15)		
				Yes		37 kHz	160 W	91	n/s	60		100% (15/15)		
												Transcutaneous concentrated US which significantly enhances streptokinase induced thrombolysis in vivo can be delivered without concomitant tissue damage. Recanalization rate increases with treatment time.		
Rabbit Iliofemoral artery occlusion		Yes				37 kHz	160 W	91	n/s	60	Measured recanalization rate	0% (0/5)		67
					Yes	37 kHz	160 W	91	n/s	60		0% (0/10)		
		Yes			Yes	37 kHz	160 W	91	n/s	15		30% (3/10)		
		Yes			Yes	37 kHz	160 W	91	n/s	30		50% (5/10)		
		Yes			Yes	37 kHz	160 W	91	n/s	45		70% (7/10)		
		Yes			Yes	37 kHz	160 W	91	n/s	60		100% (10/10)		
												In vivo arterial clot dissolution can be achieved with IV MBs and transcutaneous US. Recanalization rate increased with treatment time.		
Rabbit Marginal ear vein occlusion HIFU	rt-PA (1 mg/kg)	Yes				1 MHz	40 W	1	5	15	Measured relative clot size at 5 h post treatment	90%		5
			Yes							15		78%		
				Yes		1 MHz	40 W	1	5	15		4%		
												rt-PA-mediated thrombolysis can be significantly enhanced when combined with noninvasive pulsed-HIFU exposures.		
Rabbit Femoral artery occlusion	SK (15,000 U/kg) bolus followed by an infusion of 15,000 U/kg/h.		Yes							120	Measured volume reduction	13% (2/15)		6
				Yes		1 MHz	2 W/cm^2	c.w.		120		53% (9/17)		
												Externally applied, low-intensity US can significantly enhance thrombolysis in a rabbit arterial model.		
Rabbit Femoral artery occlusion	rt-PA (200 µg/5 mg of lipid)	Yes			Empty ELIP	5.7 MHz	1.25 MPa	5 k	n/s	2	Measured recanalization rate at 15 min post treatment	27%		68
			Yes		rt-PA ELIP					2		60%		
				Yes	rt-PA ELIP	5.7 MHz	1.25 MPa	5 k	n/s	2		100%		
												Doppler US treatment enhances the thrombolytic effect of rt-PA loaded ELIP, resulting in earlier and more complete recanalization rates.		

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Table 2. (*continued*)

Clot model	Drug	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
Rat MCA stroke	rt-PA (1.2 mg/ animal)		Yes							32	Measured volume reduction	45% (9/20)		7
				Yes		490 kHz	.8 W/cm^2	c.w.	100	32		76.2% (16/21) Low-frequency transcranial US under appropriate conditions could be an effective and safe method of treatment for ischemic stroke.		
Rabbit Femoral artery occlusion	mt-PA (1.2 mg/ animal)		Yes							32	Measured recanalization rate	16.7% (2/12)		69
				Yes		490 kHz	.13 W/cm^2	c.w.	100	32		66.7% (6/9) Low-frequency and low-intensity transcranial US enhanced thrombolysis by mt-PA.		
Rabbit Femoral artery occlusion	SK (15,000 U/ kg) as bolus followed by an infusion of 15,000 U/ kg/h	Yes				40 kHz	.75 W/cm^2	c.w.	100	120	Measured reperfusion rate	<7%		70
			Yes							120		7%		
				Yes		40 kHz	.75 W/cm^2	c.w.	100	120		83% 40-kHz US at low intensity markedly accelerates fibrinolysis and also improves tissue perfusion and reverses acidosis, effects that would be beneficial in treatment of acute thrombosis.		
Rabbit MCA stroke HIFU	rt-PA (1.mg/ ml/kg)		Yes							120	Measured recanalization rate	100%		71
				Yes	Yes	1 MHz	20 W/cm^2 (I_{SATA})	10	10	70		100% HIFU in combination with rt-PA dissolved clots.		
Rabbit MCA stroke HIFU	rt-PA (1.mg/ ml/kg)			Yes	Yes	1 MHz	20 W/cm^2 (I_{SATA})	10	10	70	Measured recanalization rate	100% Therapeutic US in synergy with rt-PA dissolve clots.		72
Rabbit Hindlimb occlusion		Yes			Yes	1 MHz	.031 W/cm^2 (I_{SATA})	.33	.17	10	Measured recanalization rate	67%		73
		Yes			Yes	1 MHz	.031 W/cm^2 (I_{SATA})	.33	.17	20		100% Long-pulse-length US with MBs has a therapeutic effect on microvascular perfusion.		
Rabbit Femoral artery occlusion	rt-PA (30 µg/kg/ min)		Yes							74	Measured initial reflow	15%-50%		74
				Yes		1 MHz	6.3 W/cm^2 I_{SPTA}	c.w.	100	33		15%-50% Although time to initial reflow was shortened by US, it was associated with less reperfusion and more reocclusion in this model.		

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Table 2. (*continued*)

Clot model	Drug	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
		US alone	Drug alone	US + drug										
Rat MCA stroke	rt-PA (10 mg/kg)	Yes				1-3 MHz	1.7 (MI)	n/s	n/s	60	Measured recanalization rate	40%		75
		Yes			Yes	1-3 MHz	1.7 (MI)	n/s	n/s	60		59%		
			Yes							60		77%		
				Yes		1-3 MHz	1.7 (MI)	n/s	n/s	60		88%		
				Yes	Yes	1-3 MHz	1.7 (MI)	n/s	n/s	60		96%		
												Recanalization rate with rt-PA alone is better than US alone. Recanalization rate significantly increased with the combination of US + drug. Recanalization rate increased even more when US was combined with rt-PA + MBs.		
Rat Carotid artery occlusion		Yes				2 MHz	1.56 MPa	150	5	30	Measured plasma D-dimer concentrations	1.70 µg/mL		76
		Yes			Yes (Nsib)	2 MHz	1.56 MPa	150	5	30		2.31 µg/mL		
		Yes			Yes (Abib)	2 MHz	1.56 MPa	150	5	30		3.91 µg/mL		
												US in combination with abciximab immunobubbles (Abib) induces thrombolysis without lytic agents that is superior to insonation of non-specific immunobubbles (Nsib).		
Rabbit Embolic stroke	rt-PA (.8-0.9 mg/kg)	Yes				1 MHz	.8 W/cm^2 (I_{SATA})	100	20	60	Measured infarct volume	1%	56% ICH	77
				Yes		1 MHz	.8 W/cm^2 (I_{SATA})	100	20	60		.13%	61% ICH	
		Yes			Yes	1 MHz	.8 W/cm^2 (I_{SATA})	100	20	60		.2%	19% ICH	
				Yes	Yes	1 MHz	.8 W/cm^2 (I_{SATA})	100	20	60		.09%	26% ICH	
												The ability of MBs to reduce rt-PA requirements may lead to lower rates of hemorrhage in human stroke treatment.		
Rabbit Embolic stroke	rt-PA (.9 mg/kg)		Yes							60	Measured infarct volume	2.2%	45% ICH	78
				Yes		1 MHz	.8 W/cm^2	n/s	20	60		1.7%	50% ICH	
		Yes			Yes	1 MHz	.8 W/cm^2	n/s	20	60		.8%	36% ICH	
												Sonothrombolysis without rt-PA using MBs is effective in decreasing infarct volumes.		

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Table 2. (*continued*)

Clot model	Drug	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
Rabbit Embolic stroke	rt-PA (.9 mg/kg)	Yes				1 MHz	.8 W/cm^2	n/s	20	60	Measured infarct volume	.97%	56% ICH	79
			Yes							60		.14%	48% ICH	
				Yes		1 MHz	.8 W/cm^2	n/s	20	60		.15%	73% ICH	
		Yes			Yes	1 MHz	.8 W/cm^2	n/s	20	60		.20%	19% ICH	
				Yes	Yes	1 MHz	.8 W/cm^2	n/s	20	60		.10% Treatment with MB + US following embolization decreased the incidence of ICH and efficacy was similar to tPA in reducing infarct volume.	36% ICH	
Rabbit Iliofemoral artery occlusion	SK (25,000 U/kg))	Yes				20 kHz	1.5 W/cm^2	n/s	n/s	60	Measured patency rate	0% (0/6)		80
			Yes							60		6% (1/17)		
				Yes		37 kHz	160 W	n/s	n/s	60		100% (15/15)		
				Yes	Yes	20 kHz	1.5 W/cm^2	n/s	n/s	60		87% (13/15)		
		Yes			Yes	20 kHz	1.5 W/cm^2	n/s	n/s	60		76% (13/17)		
		Yes			Yes	37 kHz	160 W	n/s	n/s	60		Noninvasive transcutaneous US can greatly enhance the effect of clot dissolution with thrombolytic drugs and/or MBs.		

Abib, abciximab immunobubbles; c.w., continuous wave; DF, duty factor; ELIP, echogenic liposomes; Freq., frequency; HIFU, high-intensity focused ultrasound; IV, intravenous; MBs, microbubbles; MCA, middle cerebral artery; mt-PA, monteplase tissue plasminogen activator; Nsib, nonspecific immunobubbles; n/s, not specified; PRF, pulse repetition frequency; Ref., references; rt-PA, recombinant tissue plasminogen activator; SK, streptokinase; US, ultrasound.

Table 3. *Clinical protocols used in sonothrombolysis*

Clot model	Drug	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
		US alone	Drug alone	US + drug										
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)			Yes (TCD)	Yes	2 MHz	n/s	n/s	n/s	120	Measured complete and partial recanalization rate	50% (6/12) 33% (4/12) MBs reached and permeated beyond occlusions with no increase in sICH suggesting the feasibility of further studies	0% sICH 0% sICH	81
Stroke patients MCA occlusion		Yes (TCD)				2 MHz	n/s	n/s	n/s	60	Measured complete recanalization rate	30% (4/12) Sonothrombolysis using 2 probes and bilateral monitoring is safe but not more effective than standard sonothrombolysis.	0% sICH	82
Stroke patients MCA occlusion		Yes (TCCD)				2 MHz	415 mW/cm^2 (I_{SPTA})	n/s	n/s	30	Measured partial recanalization rate	83% (5/6) High rate of early partial recanalization during continuous exposure to 2-MHz US without rt-PA.	0% sICH	83
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)		Yes							120	Measured complete recanalization rate	24% (9/36)	5.5% sICH	84
				Yes (TCD)		2 MHz	n/s	n/s	n/s	120		41% (15/37)	2.7% sICH	
				Yes (TCD)	Yes	2 MHz	n/s	n/s	n/s	120		55% (21/38) MBs administration induces further acceleration of US-enhanced thrombolysis.	2.6% sICH	
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)			Yes (TCCD)		2 MHz	189 mW/cm^2	n/s	n/s	60	Measured complete recanalization rate	53% (8/15)	7% sICH	85
				Yes (TCCD)	Yes	2 MHz	189 mW/cm^2	n/s	n/s	60		64% (7/11) MBs enhanced TCCD monitored rt-PA thrombolysis lead to a greater immediate clinical improvement.	9% sICH	
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)		Yes							60	Measured complete and partial recanalization rate	21.4% (3/14—complete) 0% (0/14—partial)	7% sICH	8
				Yes (TCCD)		2-4 MHz	179 mW/cm^2	n/s	n/s	60		27.3% (3/11—complete) 18.2% (2/11—artial) Transcranial TCCD with rt-PA showed a higher grade of recanalization compared with rt-PA alone.	36% sICH	
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)		Yes							120	Measured complete recanalization rate	30% (19/63)	5% sICH	9
				Yes (TCD)		2 MHz	750 mW/cm^2	n/s	n/s	120		49% (31/63) Continuous TCD augments rt-PA-induced arterial recanalization.	5% sICH	
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)			Yes (TCD)		2 MHz	n/s	n/s	n/s	120	Measured complete recanalization rate	36% (20/55) Complete recanalization within 2 h after rt-PA bolus is a feasible goal for thrombolysis given with TCD monitoring.	6% sICH	86

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Table 3. (*continued*)

Clot model	Drug	Mode: US alone	Mode: Drug alone	Mode: US + drug	MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Side effects	Ref.
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)		Yes							60	Measured complete and partial recanalization rate	11.1% (2/18—complete) 11.1% (2/18—partial)	5.6% sICH	[10]
				Yes (TCCD)		1.8 MHz	179 mW/cm^2	n/s	n/s	60		15.8% (3/19—complete) 42.1% (8/19—partial) Transcranial US in combination with rt-PA accelerates recanalization in MCA occlusion, compared with rt-PA alone	15.8%sICH	
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)		Yes							90	Measured recanalization rate (both complete and partial)	50% (6/12)	0% sICH	[87]
				Yes		300 kHz	700 mW/cm^2 (I_{SPTA})	100	5	90		29% (4/14) Low frequency US combined with rt-PA showed an increased rate of sICH.	36% sICH	
Stroke patients Proximal intracranial occlusion	rt-PA (.9 mg/kg)		Yes							90	Measured complete and partial recanalization rate	33% (4/12—complete) 25% (3/12—partial)	0% sICH	[88]
				Yes (TCD)	Yes (1.4 mL)	2 MHz	n/s	n/s	100	90		67% (8/12—complete) 17% (2/12—partial)	0% sICH	
				Yes (TCD)	Yes (2.8 mL)	2 MHz	n/s	n/s	100	90		45% (5/11—complete) 0% (0/11—partial) MBs can be safely combined with systemic rt-PA and US at a dose of 1.4 mL.	27% sICH	
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)	Yes (TCCD)				2-4 MHz	208 W/cm^2 (I_{SPPA})	5 k	.7	45	Measured complete and partial recanalization rate	97% (36/37—complete) 0% (0/37—partial)	2.7% sICH	[89]
			Yes							45		67% (10/15—complete) 7% (1/15—partial)	6.6% sICH	
				Yes (TCCD)		2-4 MHz	208 W/cm^2 (I_{SPPA})	5 k	.7	45		80% (12/15—complete) 7% (1/15—partial) Recanalization rate of continuous TCCD monitoring of MCA occlusion in combination with rt-PA was lower compared with that of TCCD monitoring alone.	6.6% sICH	
Stroke patients MCA occlusion Catheter type	rt-PA (60 mg IV + 22 mg IA infusion)		Yes	Yes		1.7 MHz	n/s	n/s	n/s	120 120	Measured recanalization rate (both complete and partial)	56% (33/59) 73% (24/33) EKOS micro-infusion catheter is a reasonable and easy-to-use tool for re-opening occluded intracranial arteries. Additionally, the delivery of intra-arterial rt-PA or other thrombolytic drugs via a standard micro-catheter remains an excellent option.	6.6% sICH 9.9% sICH	[90]

DF, duty factor; Freq., frequency; IA, intrarterial; IV, intravenous; MBs, microbubbles; MCA, middle cerebral artery; n/s, not specified; PRF, pulse repetition frequency; Ref., references; rt-PA, recombinant tissue plasminogen activator; sICH; symptomatic intracranial hemorrhage; TCD, transcranial Doppler; TCCD, transcranial color-coded duplex; US, ultrasound.

The treatment time used varied from 30 minutes to 10 minutes. In the animal and human studies, there was no need to specify the temperature. It is assumed that the specie of interest had the physiological temperature. In the human trials the modality used was the synergy of US and thrombolytic drugs. In some cases,[8,84,85,88] the synergy with MBs was used.

Discussion

The current study compiled various aspects associated with the protocols examined (in vitro, in vivo, and clinical) that are used in sonothrombolysis. The review protocols are summarized in tables providing all necessary data in terms of clot model, treatment mode, sonication parameters, evaluation method, sonothrombolysis efficacy, and side effects. In addition, the main conclusions derived from each study are presented as well. This study could be useful for future researchers in this area because they can easily find the US parameters used during sonothrombolysis. Additionally, they can make comparison among the various protocols used and the animal models used.

Although the mechanisms behind sonothrombolysis are not very clear, it is evidenced that exposure to US increases the uptake and depth of penetration of thrombolytics into clots,[19] causes additional binding sites due to reversible disaggregation of fibrin fibers,[18] and increases the binding of thrombolytic agents to fibrin.[17]

The influence of temperature on clot lysis was only investigated in vitro. In most of the experimental studies, the temperature during sonication was kept constant at 37°C, which sufficiently explains that clot lysis occurred through nonthermal mechanisms. In a few in vitro studies, the temperature at the target was not specified. Temperature played an important role on clot lysis because sonothrombolysis efficacy decreased at temperatures below the body baseline temperature of 37°C.[34] Therefore, it will be useful in the future, for all the in vitro studies to be conducted at 37°C.

In the animal studies the most common clot models used were the rabbit model (e.g., References 66-74). In the popular rabbit model, the most commonly used artery was the femoral, followed by the MCA and the carotid. The femoral model is used mostly because this artery is easily accessible. The MCA is widely used because of its relevance to brain stroke.

Different thrombolytic drugs such as UK, streptokinase, alteplase (rt-PA), and tenecteplase tissue plasminogen activator (TNK-tPA) in various concentrations have been investigated. Studies had shown that the synergy of US and any of these drugs could accelerate the thrombolytic activity of any thrombolytic used and that the extent of sonothrombolysis depended on the drug's concentration.[25,59] The most common thrombolytic drug used by the researchers was rt-PA, because it is the only thrombolytic treatment approved for acute ischemic stroke. Although rt-PA was administered in various concentrations between .1 and 300 μg/mL, the majority of the above-mentioned studies were conducted using 3.15 μg/mL rt-PA, which is the average concentration of the drug detected in human blood.

The current review shows that the thrombolytic efficacy of drug alone is better than that of US alone.[23,30,35] Therefore, we assume that US energy as a stand-alone method for clot lysis is not effective and should be applied in synergy with thrombolytic drugs to enhance sonothrombolysis. However, a few studies[43,44,46] demonstrated that using US alone (in the absence of thrombolytic drug), could achieve almost complete clot lysis within seconds. Taking into consideration the very high level of acoustic power used in their studies as well as the temperature elevation at the target that was not specified in their results, we suspect that most likely the protocols applied were under the influence of thermal mechanisms of sonothrombolysis. This suspicion was supported by the results of many other researchers,[23,30,35] who exhibited reduced thrombolytic efficacy using US alone, although prolonged exposure times were used in their studies.

Some other studies focused their research on the effect of traveling versus standing acoustic waves on clot lysis, demonstrating that traveling acoustic waves enhanced sonothrombolysis significantly more than standing waves did.[11,29] It is evident that researchers in this area should report whether standing waves are eliminated irrespective of the model used (in vitro, animal, or human).

It is well known that US frequency, as well as acoustic intensity, exerts a major effect on clot lysis. US frequencies ranged from 20 kHz to 5.0 MHz and intensities (either low or high) were employed for sonothrombolysis studies. A number of studies indicated that lower US frequencies (in the kilohertz range), are more efficient in sonothrombolysis over higher frequencies (in the MHz range) because they exhibited improved tissue penetration and greater acceleration in fibrinolysis.[16,37,38] Considering that some other studies showed that sonothrombolysis efficacy increases as the level of acoustic intensity increases,[24,26,28] it is reasonable to come to the conclusion that the thrombolytic efficacy of US waves is directly dependent on acoustic intensity and inversely dependent on frequency.

There was enough evidence from in vitro,[50] animal,[75] and clinical[84] studies indicating that the administration of MBs further enhanced the effect of US on enzymatic thrombolysis induced by thrombolytic agents. The findings of this review demonstrate that the boosting effect of MBs in clot dissolution correlated with the presence of cavitation mechanisms and most specifically with stable cavitation, which appeared to play a more important role in MB-mediated sonothrombolysis.[48,51,54] However, a study performed by Molina et al[88] showed that there is a safe

limit on the administered dose of MBs, which should not be exceeded because it is associated with an increased risk of symptomatic intracranial hemorrhage (sICH) rate. Therefore, it is recommended that MBs should be administered in combination with rt-PA at low doses (1.4 mL) to enhance sonothrombolysis and avoid unnecessary adverse health effects, such as sICH.

Animal studies, as well as clinical trials, have shown that the most common side effect of sonothrombolysis was sICH. Apart from the use of high dose of MBs, the application of low-frequency US might have increased the rate of sICH. The TRanscranial low-frequency sonothrombolysis in Brain Ischemia (TRUMBI) clinical trial,[87] which was designed to treat stroke patients with transcranial 300 kHz US plus rt-PA, was ended prematurely due to a significant increase in sICH rate. Since then, low-frequency US has not been available for therapeutic purposes in clinical trials. Additionally, our investigation showed that the risk of sICH rate increases with the concentration of thrombolytic drug[63] and the level of acoustic intensity.[64,65]

The effect of time is very critical on sonothrombolysis efficacy because early recanalization is the key to therapeutic success in the treatment of vascular thrombosis. In this review, the exposure times reported in the in vitro studies varied from .5 to 240 minutes. In the animal studies, the treatment times reported ranged from .5 to 120 minutes, whereas in the clinical trials, the continuous monitoring of the patients was between 30 and 120 minutes. Several studies[1,33] showed that most of the clot mass was removed within the first 60 minutes of treatment and beyond that time the efficacy of thrombolytic treatment was decreased significantly. This phenomenon was possibly caused by the concentration of the thrombolytic drug in the blood after 60 minutes of treatment, which decreased dramatically, leading to a significant reduction on enzymatic fibrinolysis rate. Therefore, at least for in vitro or animal studies, 60 minutes of treatment must be a sufficient exposure time and should not be exceeded. The long treatment time required to remove clots using sonothrombolysis prohibits the use of this method for large occluded volumes. Perhaps, the physicians will attempt to treat critical clots, thus saving as much tissue as possible, given the long treatment time needed. To treat larger occluded volumes, a multi-element transducer technology is needed.

It is evident that the long treatment time needed (30-90 minutes) to dissolve a small amount of clot imposes a limitation of the wider use of sonothrombolysis. Because the time allowed to deliver the therapy is between 3 and 6 hours, the long treatment time needed limits the wide use of sonothrombolysis.

The analysis of the protocols used in the studies evaluated revealed that there is a lack of standardization in the recording of the ultrasonic dose. Some studies reported acoustic pressure, some other studies reported power, and some reported intensity.

The most common protocol used in clinical trials so far for the treatment of patients with acute ischemic stroke due to occlusion of the MCA was the continuous monitoring of the patients with high-frequency (2 MHz) low-intensity (<750 mW/cm^2) diagnostic transcranial US in combination with rt-PA.[83,85] Using this protocol, higher recanalization rates were exhibited compared with those with rt-PA alone.

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