



City Research Online

City, University of London Institutional Repository

Citation: Papadopoulos, N., Kyriacou, P. A. and Damianou, C. (2017). Review of Protocols Used in Ultrasound Thrombolysis. *Journal of Stroke and Cerebrovascular Diseases*, 26(11), pp. 2447-2469. doi: 10.1016/j.jstrokecerebrovasdis.2017.07.032

This is the accepted version of the paper.

This version of the publication may differ from the final published version.

Permanent repository link: <http://openaccess.city.ac.uk/18276/>

Link to published version:

<http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2017.07.032>

Copyright and reuse: City Research Online aims to make research outputs of City, University of London available to a wider audience. Copyright and Moral Rights remain with the author(s) and/or copyright holders. URLs from City Research Online may be freely distributed and linked to.

City Research Online:

<http://openaccess.city.ac.uk/>

publications@city.ac.uk

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 parameters, evaluation method, thrombolysis outcome, side effects, and conclusions. *Conclusions:* The aim of this review was to give an overview of the different protocols used so far in the field of sonothrombolysis and investigate the impact of several 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/cm² to 193 W/cm². 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			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
Human	rt-PA (3000 IU/mL)	37		Yes								Measured volume reduction	33% 50%	1
					Yes	Yes	1 MHz	1.75 W/cm ² I _{SATA}	Intermittent	50	50			
Human	rt-PA (1 µg/mL)	37		Yes								Measured volume reduction	8% 17% 20%	2
					Yes	Yes	27 kHz	1 W/cm ²	70	10	60			
Human	rt-PA (3000 IU/mL)	36		Yes								Measured volume reduction	22.7% 49% (Traveling) 34.8% (Standing)	11
					Yes	Yes	2 MHz	1.2 W/cm ²	1, 10, 100, 1000	50	60			
Human	rt-PA (3000 IU/mL)	37	Yes									Measured volume reduction	25.3% 19.9%	3
					Yes	Yes	1.95 MHz	1.75 W/cm ² I _{SATA}	1	50	60			
Human	rt-PA (3.15 µg/mL)	37		Yes								Measured lytic rate	.5 µm/min 3.4 µm/min US treatment + rt-PA significantly enhanced the mean lytic rate (580% change), compared with rt-PA treatment alone.	12
				Yes	Yes	120 kHz	.35 MPa	1667	80	30				
Human	rt-PA (3.15 µg/mL)	37		Yes								Measured lytic rate	7 µm/min 15, 25, 50, 65 µm/min The lytic efficacy of clots exposed to rt-PA and US increases with increasing DF.	13
				Yes	Yes	120 kHz	.35 MPa	1667	10, 20, 50, 80	30				
Porcine	rt-PA (107 µg/mL)	37		Yes								Measured volume reduction	12% 19.1% 25%	4
					Yes	Yes	120 kHz	.35 MPa	1.7 k	80	30			
					Yes	Yes	1 MHz	.35 MPa	1.7 k	100	30			
				Yes	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.		

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
Human	rt-PA (10 µg/mL)	37		Yes	Yes		120 kHz	.35 MPa	1667	50	30	Measured volume reduction	16%	14
Human	rt-PA (3.15 µg/mL)	37		Yes	Yes		120 kHz	.35 MPa	1667	50	30	Measured volume reduction	31%	15
				Yes	Yes	Yes (t-ELIP)	120 kHz	.35 MPa	1667	50	30		71%	
				Yes	Yes	Yes (t-ELIP)	120 kHz	.35 MPa	1667	50	30		48%	
Human	rt-PA (.5-3.15 µg/mL)	37		Yes	Yes		120 kHz	.18 MPa	1667	50	30	Measured volume reduction	38%-44%	16
				Yes	Yes		2 MHz	.47 MPa	10.5 k	13	30		50%-70%	
				Yes	Yes								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	Yes		1 MHz	4 W/cm ²	c.w.	100	240	Measured uptake rate	8.2% 15.5% Exposure to US increases uptake of rt-PA into clots and also results in deeper penetration.	19
Human	rt-PA (2 µg/mL)			Yes	Yes		300 kHz	.07 W/cm ²	c.w.	100	60	Measured fibrin degradation product, D-dimer (FDP-DD)	957 ng/mL 1669 ng/mL	20
				Yes	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	Yes		1 MHz	2.5 W/cm ²	c.w.	100	60	Measured volume reduction	18.7% 52.5% c.w. US at 1 MHz and an intensity of 2.5 W/cm ² accelerates urokinase-induced thrombolysis and reperfusion.	21

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
Human	SK (5000 U/mL)	37		Yes							65	Measured reperfusion rate	100%	22
					Yes	Yes	170 kHz	.5 W/cm ² (ISATA)	c.w.	100	34		100%	
Human	rt-PA (10 µg/mL)	37	Yes	Yes							39	Measured volume reduction	100%	23
					Yes	Yes	1 MHz	1 W/cm ² (ISATA)	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	rt-PA (1.0 µg/mL)	37	Yes	Yes							60	Measured volume reduction	12.8%	24
					Yes	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	Yes							30	Measured volume reduction	18%	25
					Yes	Yes	1 MHz	2.2 W/cm ²	n/s	n/s	30		19%, 44%, 50%	
	SK (50, 250, 2000 µg/mL)	Yes	Yes								30		41%, 55%, 61%	
				Yes	Yes	1 MHz	2.2 W/cm ²	n/s	n/s	30	26%			
Human	rt-PA (1.0 µg/mL)	37	Yes	Yes							60	Measured volume reduction	23%	26
					Yes	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						25		26.7% US waves accelerate rt-PA-induced thrombolysis and reperfusion.	

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
Human	rt-PA (1 µg/mL)	37		Yes										28
					Yes		40 kHz	.25 W/cm ²	c.w.	100	60, 120	Measured volume reduction	13%, 37%	
					Yes		40 kHz	.75, 1, 1.5 W/cm ²	c.w.	100	60, 120		39%, 93%	
					Yes						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										29
				Yes	Yes		1 MHz	1.2 W/cm ²	c.w.	100	60	Measured volume reduction	23.8%	
				Yes	Yes						60		34.8 (Standing)	
				Yes	Yes		2 MHz	1.2 W/cm ²	c.w.	100	60		11.3%	
													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											30
				Yes			20 kHz	.35 W/cm ²	c.w.	100	10	Measured volume reduction	41.8%	
					Yes		20 kHz	.35 W/cm ²	c.w.	100	20		49.1%	
					Yes						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										31
				Yes + Epf.							30	Measured volume reduction	15.6%	
					Yes		120 kHz	.18 MPa	1667	80	30		28%	
					Yes + Epf.		120 kHz	.18 MPa	1667	80	30		44.4%	
													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										32
					Yes		211.5 kHz	.25 W/cm ²	c.w.	100	720	Measured volume reduction	43%	
											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
			Yes				33.3 kHz	.5 W/cm ²	n/s	n/s	60, 180	Measured volume reduction	39.96%, 44.09%	
							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.	

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.			
			US alone	Drug alone	US + drug												
Human	rt-PA (3.15 µg/mL)	33, 37	Yes	Yes	Yes		120 kHz	3.2 W/cm ²	1667	80	30	Measured volume reduction	7.5%, 7.2%	34			
																8.6%, 12.4%	
							120 kHz	3.2 W/cm ²	1667	80	30		21.2%, 22.7%				
Human through temporal bone	rt-PA (10 µg/mL)	37	Yes	Yes	Yes		1.8 MHz	1.6 MI	n/s	n/s	60	Measured volume reduction	41% 70.8%	35			
																78.7%	
							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	Yes			220 kHz	111 W/cm ²	2.5	50	.5	Measured volume reduction	76.1% (Flow)	36			
							220 kHz	111 W/cm ²	2.5	50			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	Yes		48 kHz	5-6 kPa	n/s	n/s	60	Measured volume reduction after incubation	40.6%	37			
													Yes		Yes	60, 120	59.2%
													Yes		Yes	60, 120	8.9%, 46.7%
Bovine through skull flow system	rt-PA (100 µg/mL)	37		Yes	Yes		1 MHz	.35 W/cm ² (I _{SPTP})	16 k	41.6	29.3	Measured recanalization rate	100%	38			
													Yes		Yes	17.1	100%
													Yes		Yes	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	Yes		1 MHz	.35 W/cm ² (I _{SPTP})	16,000	41.6	30	Measured recanalization rate	30%	39			
													Yes		Yes	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%	40			

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
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		2 MHz TCCD	.179 W/cm ²	n/s	n/s	60	Measured volume reduction	36.7%	42	
					Yes		2 MHz TCD	.457 W/cm ²	n/s	n/s		60		40.8%
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	Yes	1 MHz	4 W/cm ² (I _{SATA})	c.w.	100	60		21.8% at 0 atm 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		120 kHz	.15 MPa	1667	80	30	Measured volume reduction	13%	48	
					Yes		.24 MPa	1667	80	30		13.7% (<SC)		
					Yes		.36 MPa	1667	80	30		26% (SC)		
											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.			

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
Human catheter type flow system	rt-PA (300 µg/mL)	37		Yes										49
						Yes	1.7 MHz	62.46 W/cm ² (ISPPA)	30	8.5	10	Measured volume reduction	13.6% 21.4%	
						Yes	1.7 MHz	180.02 W/cm ² (ISPPA)	40	4	10		46.1% Thrombolysis efficiency increases with intensity.	
Human	rt-PA (96 µg/mL)	37	Yes	Yes		Yes	120 kHz	.32 MPa	1667	80	30	Measured volume reduction	6.3%	50
						Yes	120 kHz	.32 MPa	1667	80	30		13.0%	
						Yes	120 kHz	.32 MPa	1667	80	30		16.0%	
						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		Yes	500 kHz	.7 W/cm ²	c.w.	100	1	Measured volume reduction	25.8%	49
							500 kHz	.7 W/cm ²	c.w.	100	1		24.2%	
						Yes	500 kHz	.7 W/cm ²	c.w.	100	1		24.9%	
						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		Yes	1.7 MHz	4.9 W/cm ² ISATA	n/s	n/s	30	Measured volume reduction	.95%	51
							1.7 MHz	4.9 W/cm ² ISATA	n/s	n/s	30		7.68%	
						Yes	1.7 MHz	4.9 W/cm ² ISATA	n/s	n/s	30		11.10%	
						Yes	1.7 MHz	4.9 W/cm ² ISATA	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	Yes		Yes	2 MHz	455 mW/cm ² ISPTA	5000	n/s	30	Measured volume reduction	6.1%	52
							2 MHz	455 mW/cm ² ISPTA	5000	n/s	30		10.9%	
						Yes	2 MHz	455 mW/cm ² ISPTA	5000	n/s	30		13.1%	
						Yes	2 MHz	455 mW/cm ² ISPTA	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		Yes (3 µm)	1 MHz	.1 W/cm ²	100	20	30	Measured volume reduction	18%	53
						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/cm ²) than with 1 µm MBs (2.0 W/cm ²).	
Porcine flow system	rt-PA (7.1 µg/mL)	37		Yes										54
				Yes		Yes	120 kHz	.44 MPa	c.w.	100	30	Measured volume reduction	29%	
					Yes	120 kHz	.44 MPa	c.w.	100	30	34%	83% (SC) SC nucleated by an infusion of MBs enhances rt-PA thrombolysis without apparent treatment-related damage.		

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.
			US alone	Drug alone	US + drug									
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								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							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	Yes	120 kHz	.44 MPa	Inter.	62.5	30		.8%-2%/min	
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.		

(continued on next page)

Table 1. (continued)

Clot model	Drug	Temp. (°C)	Mode			MBs	Freq.	Output	PRF (Hz)	DF (%)	Time (min)	Evaluation method	Main conclusion	Ref.	
			US alone	Drug alone	US + drug										
Porcine FUS flow system	rt-PA (3.5 µg/mL)	37	Yes	Yes		Yes	1 MHz	20 W	100	10	30	Measured volume reduction	31%	61	
							Yes	1 MHz	20 W	100	10		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	Yes		Yes	.6 MHz	60 W	100	10	30	Measured volume reduction	29.2%	62	
							Yes	1 MHz	20-60 W	100	10		30		45.8%
							Yes	1 MHz	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% 41%, 55% 28%, 40% 45%, 50%	1	
				Yes	1 MHz	1.75 W/cm ²	Intermittent	50	50, 200					
	rt-PA (2 mg)		Yes		1 MHz	1.75 W/cm ²	Intermittent	50	50, 200					
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					
	rt-PA (10 mg/kg)			Yes	25.6 kHz	.6 W/cm ²	n/s	20	60					
Rabbit Embolic stroke HIFU		Yes			1.5 MHz	255 W	1	.1	.33	Measured reperfusion rate	0% (0/3) 50% (2/4) 70% (5/7)	20% ICH	64	
		Yes			1.5 MHz	415 W	1	.1	.33					
		Yes			1.5 MHz	550 W	1	.1	.33					
Rabbit MCA Stroke HIFU		Yes		Yes	1.5 MHz	88-137 W	1	.1	.33	Measured recanalization rate	78% (7/9) 50% (1/2)	22% ICH	65	
		Yes		Yes	1.5 MHz	88 W	10	.1	.33					
Rabbit Femoral artery occlusion HIFU		Yes			1.51 MHz	185 W	1	.1	.33	Measured flow restoration rate	0% (0/5) 50% (1/2) 63% (5/8)	13% ICH	48	
		Yes			1.51 MHz	215 W	1	.1	.33					
		Yes			1.51 MHz	300 W	1	.1	.33					

(continued on next page)

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										
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)		
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)		
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			1 MHz	40 W	1	5	15		78%		
Rabbit Femoral artery occlusion	SK (15,000 U/kg) bolus followed by an infusion of 15,000 U/kg/h.		Yes								Measured volume reduction	4%		6
				Yes		1 MHz	2 W/cm ²	c.w.				120		
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	78%		68
			Yes		rt-PA					2		60%		
				Yes	ELIP	5.7 MHz	1.25 MPa	5 k	n/s	2		100%		
					rt-PA ELIP							Doppler US treatment enhances the thrombolytic effect of rt-PA loaded ELIP, resulting in earlier and more complete recanalization rates.		

(continued on next page)

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 (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	Yes		40 kHz	.75 W/cm ²	c.w.	100	120		7% 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			1 MHz	6.3 W/cm ² I _{SPTA}	c.w.	100	33	Measured initial reflow	15%-50% 15%-50% Although time to initial reflow was shortened by US, it was associated with less reperfusion and more reocclusion in this model.	74	

(continued on next page)

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%				
<p>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.</p>															
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			
<p>US in combination with abciximab immunobubbles (Abib) induces thrombolysis without lytic agents that is superior to insonation of non-specific immunobubbles (Nsib).</p>															
Rabbit Embolic stroke	rt-PA (.8-0.9 mg/ kg)	Yes				1 MHz	.8 W/cm ² (ISATA)	100	20	60	Measured infarct volume	1%	56% ICH	77	
				Yes			1 MHz	.8 W/cm ² (ISATA)	100	20		60	.13%		61% ICH
			Yes		Yes		1 MHz	.8 W/cm ² (ISATA)	100	20		60	.2%		19% ICH
				Yes	Yes		1 MHz	.8 W/cm ² (ISATA)	100	20		60	.09%		26% ICH
<p>The ability of MBs to reduce rt-PA requirements may lead to lower rates of hemorrhage in human stroke treatment.</p>															
Rabbit Embolic stroke	rt-PA (.9 mg/kg)		Yes							60	Measured infarct volume	2.2%	45% ICH	78	
		Yes		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			
<p>Sonothrombolysis without rt-PA using MBs is effective in decreasing infarct volumes.</p>															

(continued on next page)

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											
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%	36% ICH		
												Treatment with MB + US following embolization decreased the incidence of ICH and efficacy was similar to tPA in reducing infarct volume.			
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) 41% (15/37) 55% (21/38) MBs administration induces further acceleration of US-enhanced thrombolysis.	5.5% sICH 2.7% sICH 2.6% sICH	84
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) 64% (7/11) MBs enhanced TCCD monitored rt-PA thrombolysis lead to a greater immediate clinical improvement.	7% sICH 9% sICH	85
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) 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.	7% sICH	8
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)		Yes							120	Measured complete recanalization rate	30% (19/63) 49% (31/63) Continuous TCD augments rt-PA-induced arterial recanalization.	5% sICH 5% sICH	9
Stroke patients MCA occlusion	rt-PA (.9 mg/kg)			Yes (TCD)	Yes	2 MHz	750 mW/cm ²	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

(continued on next page)

Table 3. (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										
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) 29% (4/14)	0% sICH 36% sICH	87
				Yes		300 kHz	700 mW/cm ² (ISPTA)	100	5	90		Low frequency US combined with rt-PA showed an increased rate of 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)								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 (ISPPA)	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 clinical⁸⁴ 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 al⁸⁸ 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 drug⁶³ 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/cm²) 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.

Acknowledgment: This work was supported by a starting grant of professor Christakis Damianou at Cyprus University of Technology.

References

1. Lauer CG, Burge R, Tang DB, et al. Effect of ultrasound on tissue-type plasminogen activator-induced thrombolysis. *Circulation* 1992;86:1257-1264.
2. Suchkova V, Carstensen EL, Francis CW. Ultrasound enhancement of fibrinolysis at frequencies of 27 to 100 kHz. *Ultrasound Med Biol* 2002;28:377-382.
3. Devic-Kuhar B, Pfaffenberger S, Gherardini L, et al. Ultrasound affects distribution of plasminogen and tissue-type plasminogen activator in whole blood clots in vitro. *Thromb Haemost* 2004;92:980-985.
4. Holland CK, Vaidya SS, Datta S, et al. Ultrasound-enhanced tissue plasminogen activator thrombolysis in an in vitro porcine clot model. *Thromb Res* 2008;121:663-673.
5. Stone MJ, Frenkel V, Dromi S, et al. Pulsed-high intensity focused ultrasound enhanced tPA mediated thrombolysis in a novel in vivo clot model, a pilot study. *Thromb Res* 2007;121:193-202.
6. Riggs PN, Francis CW, Bartos SR, et al. Ultrasound enhancement of rabbit femoral artery thrombolysis. *Cardiovasc Surg* 1997;5:201-207.
7. Saguchi T, Onoue H, Urashima M, et al. Effective and safe conditions of low-frequency transcranial ultrasonic thrombolysis for acute ischemic stroke: neurologic and histologic evaluation in a rat middle cerebral artery stroke model. *Stroke* 2008;39:1007-1011.
8. Eggers J, Koch B, Meyer K, et al. Effect of ultrasound on thrombolysis of middle cerebral artery occlusion. *Ann Neurol* 2003;53:797-800.
9. Alexandrov AV, Molina CA, Grotta JC, et al. Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. *N Engl J Med* 2004;351:2170-2178.
10. Eggers J, König IR, Koch B, et al. Sonothrombolysis with transcranial color-coded sonography and recombinant tissue-type plasminogen activator in acute middle cerebral artery main stem occlusion: results from a randomized study. *Stroke* 2008;39:1470-1475.
11. Pfaffenberger S, Devic-Kuhar B, El-Rabadi K, et al. 2 MHz ultrasound enhances t-PA-mediated thrombolysis: comparison of continuous versus pulsed ultrasound and standing versus travelling acoustic waves. *Thromb Haemost* 2003;89:583-589.
12. Cheng JY, Shaw GJ, Holland CK. In vitro microscopic imaging of enhanced thrombolysis with 120-kHz ultrasound in a human clot model. *Acoust Res Lett Online* 2005;6:25.
13. Meunier JM, Holland CK, Lindsell CJ, et al. Duty cycle dependence of ultrasound enhanced thrombolysis in a

- human clot model. *Ultrasound Med Biol* 2007;33:576-583.
14. Shaw GJ, Meunier JM, Lindsell CJ, et al. Tissue plasminogen activator concentration dependence of 120 kHz ultrasound-enhanced thrombolysis. *Ultrasound Med Biol* 2008;34:1783-1792.
 15. Shaw GJ, Meunier JM, Huang S-L, et al. Ultrasound-enhanced thrombolysis with tPA-loaded echogenic liposomes. *Thromb Res* 2009;124:306-310.
 16. Meunier JM, Holland CK, Porter TM, et al. Combination treatment with rt-PA is more effective than rt-PA alone in an in vitro human clot model. *Curr Neurovasc Res* 2011;8:305-312.
 17. Siddiqi F, Odriljin TM, Fay PJ, et al. Binding of tissue-plasminogen activator to fibrin: effect of ultrasound. *Blood* 1998;91:2019-2025.
 18. Braaten JV, Goss RA, Francis CW. Ultrasound reversibly disaggregates fibrin fibers. *Thromb Haemost* 1997;78:1063-1068.
 19. Francis CW, Blinc A, Lee S, et al. Ultrasound accelerates transport of recombinant tissue plasminogen activator into clots. *Ultrasound Med Biol* 1995;21:419-424.
 20. Kimura M, Iijima S, Kobayashi K, et al. Evaluation of the thrombolytic effect of tissue-type plasminogen activator with ultrasonic irradiation: in vitro experiment involving assay of the fibrin degradation products from the clot. *Biol Pharm Bull* 1994;17:126-130.
 21. Harpaz D, Chen X, Francis CW, et al. Ultrasound accelerates urokinase-induced thrombolysis and reperfusion. *Am Heart J* 1994;127:1211-1219.
 22. Olsson SB, Johansson B, Nilsson AM, et al. Enhancement of thrombolysis by ultrasound. *Ultrasound Med Biol* 1994;20:375-382.
 23. Frenkel V, Oberoi J, Stone MJ, et al. Pulsed high-intensity focused ultrasound enhances thrombolysis in an in vitro model. *Radiology* 2006;239:86-93.
 24. Francis CW, Onundarson PT, Carstensen EL, et al. Enhancement of fibrinolysis in vitro by ultrasound. *J Clin Invest* 1992;90:2063-2068.
 25. Luo H, Steffen W, Cercek B, et al. Enhancement of thrombolysis by external ultrasound. *Am Heart J* 1993;125:1564-1569.
 26. Blinc A, Francis CW, Trudnowski JL, et al. Characterization of ultrasound-potentiated fibrinolysis in vitro. *Blood* 1993;81:2636-2643.
 27. Harpaz D, Chen X, Francis CW, et al. Ultrasound enhancement of thrombolysis and reperfusion in vitro. *J Am Coll Cardiol* 1993;21:1507-1511.
 28. Suchkova V, Siddiqi FN, Carstensen EL, et al. Enhancement of fibrinolysis with 40-kHz ultrasound. *Circulation* 1998;98:1030-1035.
 29. Devcic-Kuhar B, Pfaffenberger S, Gröschl M, et al. In vitro thrombolysis enhanced by standing and travelling ultrasound wave fields. *Ultrasound Med Biol* 2002;28:1181-1187.
 30. Nedelmann M, Eicke BM, Lierke EG, et al. Low-frequency ultrasound induces nonenzymatic thrombolysis in vitro. *J Ultrasound Med* 2002;21:649-656.
 31. Meunier JM, Holland CK, Pancioli AM, et al. Effect of low frequency ultrasound on combined rt-PA and eptifibatide thrombolysis in human clots. *Thromb Res* 2009;123:528-536.
 32. Akiyama M, Ishibashi T, Yamada T, et al. Low-frequency ultrasound penetrates the cranium and enhances thrombolysis in vitro. *Neurosurgery* 1998;43:828-832.
 33. Behrens S, Daffertshofer M, Spiegel D, et al. Low-frequency, low-intensity ultrasound accelerates thrombolysis through the skull. *Ultrasound Med Biol* 1999;25:269-273.
 34. Shaw GJ, Bavani N, Dhamija A, et al. Effect of mild hypothermia on the thrombolytic efficacy of 120 kHz ultrasound enhanced thrombolysis in an in-vitro human clot model. *Thromb Res* 2006;117:603-608.
 35. Eggers J, Ossadnik S, Seidel G. Enhanced clot dissolution in vitro by 1.8-MHz pulsed ultrasound. *Ultrasound Med Biol* 2009;35:523-526.
 36. Hölscher T, Fisher D, Raman R. Noninvasive transcranial clot lysis using high intensity focused ultrasound. *J Neurol Neurophysiol* 2011;01:1-6.
 37. Tachibana K. Enhancement of fibrinolysis with ultrasound energy. *J Vasc Interv Radiol* 1992;3:299-303.
 38. Behrens S, Spengos K, Daffertshofer M, et al. Transcranial ultrasound-improved thrombolysis: Diagnostic vs. therapeutic ultrasound. *Ultrasound Med Biol* 2001;27:1683-1689.
 39. Spengos K, Behrens S, Daffertshofer M, et al. Acceleration of thrombolysis with ultrasound through the cranium in a flow model. *Ultrasound Med Biol* 2000;26:889-895.
 40. Hölscher T, Raman R, Fisher DJ, et al. Effects of varying duty cycle and pulse width on high-intensity focused ultrasound (HIFU)-induced transcranial thrombolysis. *J Ther ultrasound* 2013;1:18.
 41. Saletes I, Bruno G, Auboiron V, et al. In vitro demonstration of focused ultrasound thrombolysis using bifrequency excitation. *Biomed Res Int* 2014;2014.
 42. Roessler FC, Teichert A, Ohlrich M, et al. Development of a new clot formation protocol for standardized in vitro investigations of sonothrombolysis. *J Neurosci Methods* 2014;237:26-32.
 43. Durst C, Monteith S, Sheehan J, et al. Optimal imaging of in vitro clot sonothrombolysis by MR-guided focused ultrasound. *J Neuroimaging* 2013;23:187-191.
 44. Ahadi G, Welch CS, Grimm MJ, et al. Transcranial sonothrombolysis using high-intensity focused ultrasound: impact of increasing output power on clot fragmentation. *J Ther Ultrasound* 2013;1:22.
 45. Rosenschein U, Furman V, Kerner E, et al. Ultrasound imaging-guided noninvasive ultrasound thrombolysis: preclinical results. *Circulation* 2000;102:238-245.
 46. Wright C, Hynynen K, Goertz D. In vitro and in vivo high-intensity focused ultrasound thrombolysis. *Invest Radiol* 2012;47:217-225.
 47. Everbach EC, Francis CW. Cavitation mechanisms in ultrasound-accelerated thrombolysis at 1 MHz. *Ultrasound Med Biol* 2000;26:1153-1160.
 48. Datta S, Coussios CC, McAdory LE, et al. Correlation of cavitation with ultrasound enhancement of thrombolysis. *Ultrasound Med Biol* 2006;32:1257-1267.
 49. Zenitani T, Suzuki R, Maruyama K, et al. Accelerating effects of ultrasonic thrombolysis with bubble liposomes. *J Med Ultrason* 2008;35:5-10.
 50. Datta S, Coussios CC, Ammi AY, et al. Ultrasound-enhanced thrombolysis using Definity® as a cavitation nucleation agent. *Ultrasound Med Biol* 2008;34:1421-1433.
 51. Prokop AF, Soltani A, Roy RA. Cavitation mechanisms in ultrasound-accelerated fibrinolysis. *Ultrasound Med Biol* 2007;33:924-933.
 52. Cintas P, Nguyen F, Boneu B, et al. Enhancement of enzymatic fibrinolysis with 2-MHz ultrasound and microbubbles. *J Thromb Haemost* 2004;2:1163-1166.
 53. Borrelli MJ, O'Brien WD, Hamilton E, et al. Influences of microbubble diameter and ultrasonic parameters on in vitro sonothrombolysis efficacy. *J Vasc Interv Radiol* 2012;23:1677-1684.

54. Hitchcock KE, Ivancevich NM, Haworth KJ, et al. Ultrasound-enhanced rt-PA thrombolysis in an ex vivo porcine carotid artery model. *Ultrasound Med Biol* 2011;37:1240-1251.
55. Bohren Y, Gaud E, Arditi M, et al., In vitro sonothrombolysis of human blood clots with BR38 microbubbles, in AIP Conference Proceedings, vol. 1503, pp. 244-249, 2012.
56. Kim JS, Leeman JE, Kagemann L, et al. Volumetric quantification of in vitro sonothrombolysis with microbubbles using high-resolution optical coherence tomography. *J Biomed Opt* 2012;17:070502.
57. Leeman JE, Kim JS, Yu FTH, et al. Effect of acoustic conditions on microbubble-mediated microvascular sonothrombolysis. *Ultrasound Med Biol* 2012;38:1589-1598.
58. Wu J, Xie F, Kumar T, et al. Improved sonothrombolysis from a modified diagnostic transducer delivering impulses containing a longer pulse duration. *Ultrasound Med Biol* 2014;40:1545-1553.
59. Bader KB, Gruber MJ, Holland CK. Shaken and stirred: mechanisms of ultrasound-enhanced thrombolysis. *Ultrasound Med Biol* 2015;41:187-196.
60. Petit B, Bohren Y, Gaud E, et al. Sonothrombolysis: the contribution of stable and inertial cavitation to clot lysis. *Ultrasound Med Biol* 2015;41:1402-1410.
61. Papadopoulos N, Damianou C. In vitro evaluation of focused ultrasound-enhanced TNK-tissue plasminogen activator-mediated thrombolysis. *J Stroke Cerebrovasc Dis* 2016;16:1052-3057.
62. 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.
63. Daffertshofer M, Huang Z, Fatar M, et al. Efficacy of sonothrombolysis in a rat model of embolic ischemic stroke. *Neurosci Lett* 2004;361:115-119.
64. Burgess A, Huang Y, Waspe AC, et al. High-intensity focused ultrasound (HIFU) for dissolution of clots in a rabbit model of embolic stroke. *PLoS ONE* 2012;7:e42311.
65. Pajek D, Burgess A, Huang Y, et al. 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.
66. Huai L, Birnbaum Y, Fishbein MC, et al. Enhancement of thrombolysis in vivo without skin and soft tissue damage by transcutaneous ultrasound. *Thromb Res* 1998;89:171-177.
67. Birnbaum Y, Luo H, Nagai T, et al. Noninvasive in vivo clot dissolution without a thrombolytic drug: recanalization of thrombosed iliofemoral arteries by transcutaneous ultrasound combined with intravenous infusion of microbubbles. *Circulation* 1998;97:130-134.
68. Laing ST, Moody M, Smulevitz B, et al. Ultrasound-enhanced thrombolytic effect of tissue plasminogen activator-loaded echogenic liposomes in an in vivo rabbit aorta thrombus model—brief report. *Arterioscler Thromb Vasc Biol* 2011;31:1357-1359.
69. Ishibashi T, Akiyama M, Onoue H, et al. Can transcranial ultrasonication increase recanalization flow with tissue plasminogen activator? *Stroke* 2002;33:1399-1404.
70. Suchkova VN, Baggs RB, Francis CW. Effect of 40-kHz ultrasound on acute thrombotic ischemia in a rabbit femoral artery thrombosis model: enhancement of thrombolysis and improvement in capillary muscle perfusion. *Circulation* 2000;101:2296-2301.
71. Damianou C, Hadjisavvas V, Mylonas N, et al. MRI-guided sonothrombolysis of rabbit carotid artery. *J Stroke Cerebrovasc Dis* 2014;23.
72. Damianou C, Mylonas N, Ioannides K. Sonothrombolysis in combination with thrombolytic drugs in a rabbit model using MRI-guidance. *Engineering* 2013;5:352-356.
73. Pacella JJ, Brands J, Schnatz FG, et al. Treatment of microvascular micro-embolization using microbubbles and long-tone-burst ultrasound: an in vivo study. *Ultrasound Med Biol* 2015;41:456-464.
74. Kornowski R, Meltzer RS, Chernine A, et al. Does external ultrasound accelerate thrombolysis? Results from a rabbit model. *Circulation* 1994;89:339-344.
75. Nedelmann M, Ritschel N, Doenges S, 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.
76. Alonso A, Dempfle CE, Della Martina A, et al. In vivo clot lysis of human thrombus with intravenous abciximab immunobubbles and ultrasound. *Thromb Res* 2009;124:70-74.
77. Brown AT, Flores R, Hamilton E, et al. Microbubbles improve sonothrombolysis in vitro and decrease hemorrhage in vivo in a rabbit stroke model. *Invest Radiol* 2011;46:202-207.
78. Culp WC, Flores R, Brown AT, et al. Successful microbubble sonothrombolysis without tissue-type plasminogen activator in a rabbit model of acute ischemic stroke. *Stroke* 2011;42:2280-2285.
79. Flores R, Hennings LJ, Lowery JD, et al. Microbubble-augmented ultrasound sonothrombolysis decreases intracranial hemorrhage in a rabbit model of acute ischemic stroke. *Invest Radiol* 2011;46:419-424.
80. Siegel RJ, Atar S, Fishbein MC, et al. Noninvasive transcutaneous low frequency ultrasound enhances thrombolysis in peripheral and coronary arteries. *Echocardiography* 2001;18:247-257.
81. Alexandrov AV, Mikulik R, Ribo M, et al. A pilot randomized clinical safety study of sonothrombolysis augmentation with ultrasound-activated perflutren-lipid microspheres for acute ischemic stroke. *Stroke* 2008;39:1464-1469.
82. Bardon P, Kuliha M, Herzig R, et al. Safety and efficacy of sonothrombolysis using bilateral TCD monitoring by diagnostic 2 MHz probes—a pilot study. *Biomed. Pap* 2014;158:233-237.
83. Cintas P, Le Traon AP, Larrue V. High rate of recanalization of middle cerebral artery occlusion during 2-MHz transcranial color-coded Doppler continuous monitoring without thrombolytic drug. *Stroke* 2002;33:626-628.
84. Molina CA, Ribo M, Rubiera M, et al. Microbubble administration accelerates clot lysis during continuous 2-MHz ultrasound monitoring in stroke patients treated with intravenous tissue plasminogen activator. *Stroke* 2006;37:425-429.
85. Perren F, Loulidi J, Poglia D, et al. Microbubble potentiated transcranial duplex ultrasound enhances IV thrombolysis in acute stroke. *J Thromb Thrombolysis* 2008;25:219-223.
86. Alexandrov AV, Demchuk AM, Burgin WS, et al. Ultrasound-enhanced thrombolysis for acute ischemic stroke: phase I. Findings of the CLOTBUST trial. *J Neuroimaging* 2004;14:113-117.

87. Daffertshofer M, Gass A, Ringleb P, et al. 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.
88. Molina CA, Barreto AD, Tsivgoulis G, et al. Transcranial ultrasound in clinical sonothrombolysis (TUCSON) trial. *Ann Neurol* 2009;66:28-38.
89. Skoloudik D, Bar M, Skoda O, et al. Safety and efficacy of the sonographic acceleration of the middle cerebral artery recanalization: results of the pilot thrombotripsy study. *Ultrasound Med Biol* 2008;34:1775-1782.
90. IMS II Trial Investigators. The interventional management of stroke (IMS) II study. *Stroke* 2007;38:2127-2135.