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# Review of Protocols Used in Ultrasound Thrombolysis

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*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.

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## Introduction

One of the early investigators who used ultrasound (US) energy to accelerate the fibrinolytic activity of thrombolytic drugs in vitro was Lauer.<sup>1</sup> 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).<sup>2</sup> Another major goal was to test the best thrombolytic drug that enhances sonothrombolysis.<sup>3,4</sup> 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.<sup>5</sup> Additionally, different clot animal models were used which could test the various derived ultrasonic protocols.<sup>6,7</sup> Progressively, US bubbles were employed which can possibly enhance the efficacy of sonothrombolysis.<sup>5</sup> When sufficient data were collected this research was translated into clinical trials.<sup>8-10</sup> 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

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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](http://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,<sup>4,48,54,56-58</sup> the rabbit model,<sup>46,53</sup> and the bovine.<sup>45,49</sup> The intensity used ranged from .5 W/cm<sup>2</sup> to 193 W/cm<sup>2</sup>. 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.<sup>21,25,32,36</sup> 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,<sup>34</sup> 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<sup>5,6,48,64-74,78-80</sup> and the rat model.<sup>1,7,63,75</sup> 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.<sup>66,70,80</sup> 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<sup>71-73,78-80</sup> 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.<br>(°C) | Mode        |               |           | MBs      | Freq.                                       | Output                                      | PRF (Hz)     | DF<br>(%)                                                                                                                      | Time<br>(min)                                                                                                                                                                                          | Evaluation<br>method         | Main conclusion                                                                                                | Ref. |
|------------|-----------------------|---------------|-------------|---------------|-----------|----------|---------------------------------------------|---------------------------------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|----------------------------------------------------------------------------------------------------------------|------|
|            |                       |               | US<br>alone | Drug<br>alone | US + drug |          |                                             |                                             |              |                                                                                                                                |                                                                                                                                                                                                        |                              |                                                                                                                |      |
| Human      | rt-PA<br>(3000 IU/mL) | 37            |             | Yes           |           |          |                                             |                                             |              |                                                                                                                                | 50                                                                                                                                                                                                     | Measured volume<br>reduction | 33%                                                                                                            | 1    |
|            |                       |               |             |               | Yes       |          | 1 MHz                                       | 1.75 W/cm <sup>2</sup><br>I <sub>SATA</sub> | Intermittent | 50                                                                                                                             | 50                                                                                                                                                                                                     |                              | 50%                                                                                                            |      |
|            |                       |               |             |               | Yes       |          | 1 MHz                                       | 1.75 W/cm <sup>2</sup><br>I <sub>SATA</sub> | Intermittent | 50                                                                                                                             | 200                                                                                                                                                                                                    |                              | 62%                                                                                                            |      |
| Human      | rt-PA<br>(1 µg/mL)    | 37            |             |               |           |          |                                             |                                             |              |                                                                                                                                | 60                                                                                                                                                                                                     | Measured volume<br>reduction | 8%                                                                                                             | 2    |
|            |                       |               |             | Yes           |           | 27 kHz   | 1 W/cm <sup>2</sup>                         | 70                                          | 10           | 60                                                                                                                             | 17%                                                                                                                                                                                                    |                              |                                                                                                                |      |
|            |                       |               |             | Yes           |           | 40 kHz   | 1 W/cm <sup>2</sup>                         | 70                                          | 10           | 60                                                                                                                             | 20%                                                                                                                                                                                                    |                              |                                                                                                                |      |
|            |                       |               |             | Yes           |           | 100 kHz  | 1 W/cm <sup>2</sup>                         | 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<br>(3000 IU/mL) | 36            |             | Yes           |           |          |                                             |                                             |              |                                                                                                                                | 60                                                                                                                                                                                                     | Measured volume<br>reduction | 22.7%                                                                                                          | 11   |
|            |                       |               |             | Yes           |           | 2 MHz    | 1.2 W/cm <sup>2</sup>                       | cw                                          | 100          | 60                                                                                                                             | 49% (Traveling)                                                                                                                                                                                        |                              |                                                                                                                |      |
|            |                       |               |             | Yes           |           | 2 MHz    | 1.2 W/cm <sup>2</sup>                       | cw                                          | 100          | 60                                                                                                                             | 34.8% (Standing)                                                                                                                                                                                       |                              |                                                                                                                |      |
|            |                       |               |             | Yes           |           | 2 MHz    | 1.2 W/cm <sup>2</sup>                       | 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<br>(3000 IU/mL) | 37            | Yes         |               |           |          |                                             |                                             |              |                                                                                                                                | 60                                                                                                                                                                                                     | Measured volume<br>reduction | 25.3%                                                                                                          | 3    |
|            |                       |               |             | Yes           |           | 1.95 MHz | 1.2 W/cm <sup>2</sup><br>I <sub>SATA</sub>  | 1                                           | 50           | 60                                                                                                                             | 19.9%                                                                                                                                                                                                  |                              |                                                                                                                |      |
|            |                       |               |             | Yes           |           | 1.95 MHz | 1.75 W/cm <sup>2</sup><br>I <sub>SATA</sub> | 1                                           | 50           | 60                                                                                                                             | 35.2% US enhances thrombolysis by affecting the distribution of rt-PA within the clot.                                                                                                                 |                              |                                                                                                                |      |
| Human      | rt-PA<br>(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<br>(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<br>(107 µg/mL)  | 37            |             | Yes           |           |          |                                             |                                             |              |                                                                                                                                | 30                                                                                                                                                                                                     | Measured volume<br>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. |      |

(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%<br>31% 120-kHz US substantially increases the lytic efficacy of rt-PA for almost all rt-PA concentrations.                                                                                                                                        | 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 (t-ELIP) |         |                       |          |        | 30         |                                                       | 71%                                                                                                                                                                                                                                                   |      |
|                   |                       |            |          |            | Yes       | Yes (t-ELIP) | 120 kHz | .35 MPa               | 1667     | 50     | 30         |                                                       | 48%                                                                                                                                                                                                                                                   |      |
| Human             | rt-PA (.5-3.15 µg/mL) | 37         |          | Yes        |           |              |         |                       |          |        | 30         | Measured volume reduction                             | 89% The addition of 120 kHz US with t-ELIP substantially increases the rate of lysis compared with either tPA or t-ELIP alone.                                                                                                                        | 16   |
|                   |                       |            |          |            | Yes       |              | 120 kHz | .18 MPa               | 1667     | 50     | 30         |                                                       | 38%-44%                                                                                                                                                                                                                                               |      |
|                   |                       |            |          |            | Yes       |              | 2 MHz   | .47 MPa               | 10.5 k   | 13     | 30         |                                                       | 50%-70%                                                                                                                                                                                                                                               |      |
| Fibrin gel        | rt-PA (.1 µg/mL)      | 25         |          |            | Yes       |              | 1 MHz   | 2 W/cm <sup>2</sup>   | 100      | 50     |            | Measured binding ratios                               | 52%-58% Combination treatment with rt-PA is more effective than rt-PA alone in human whole blood clots.                                                                                                                                               | 17   |
| Fibrin gel        |                       |            | Yes      |            |           |              | 1 MHz   | 4 W/cm <sup>2</sup>   | c.w.     | 100    | 15         | Measured fiber density<br>Measured fiber diameter     | US exposure accelerates rt-PA binding, alters binding affinity, and increases maximum binding to polymerized fibrin                                                                                                                                   | 18   |
| Human             | rt-PA (.1 µg/mL)      | 37         |          | Yes        |           |              |         |                       |          |        | 240        | Measured uptake rate                                  | >65%                                                                                                                                                                                                                                                  | 19   |
|                   |                       |            |          |            | Yes       |              | 1 MHz   | 4 W/cm <sup>2</sup>   | c.w.     | 100    | 240        |                                                       | <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.     |      |
| Human             | rt-PA (2 µg/mL)       |            |          | Yes        |           |              |         |                       |          |        | 60         | Measured fibrin degradation product, D-dimer (FDP-DD) | 8.2%                                                                                                                                                                                                                                                  | 20   |
|                   |                       |            |          |            | Yes       |              | 300 kHz | .07 W/cm <sup>2</sup> | c.w.     | 100    | 60         |                                                       | 15.5% Exposure to US increases uptake of rt-PA into clots and also results in deeper penetration.                                                                                                                                                     |      |
|                   |                       |            |          |            | Yes       |              | 1 MHz   | .4 W/cm <sup>2</sup>  | c.w.     | 100    | 60         |                                                       | 957 ng/mL                                                                                                                                                                                                                                             |      |
| Human flow system | UK (400 U/mL)         | 37         |          | Yes        |           |              |         |                       |          |        | 60         | Measured volume reduction                             | 1669 ng/mL                                                                                                                                                                                                                                            | 21   |
|                   |                       |            |          |            | Yes       |              | 1 MHz   | 2.5 W/cm <sup>2</sup> | 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. |      |

(continued on next page)

**Table 1.** *(continued)*

| Clot model    | Drug                          | Temp.<br>(°C) | Mode        |               |           | MBs | Freq.                 | Output                              | PRF (Hz) | DF<br>(%) | Time<br>(min) | Evaluation<br>method                                                                                                                                                                                             | Main conclusion                                                                                                                                                                                     | Ref. |  |
|---------------|-------------------------------|---------------|-------------|---------------|-----------|-----|-----------------------|-------------------------------------|----------|-----------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|--|
|               |                               |               | US<br>alone | Drug<br>alone | US + drug |     |                       |                                     |          |           |               |                                                                                                                                                                                                                  |                                                                                                                                                                                                     |      |  |
| Human         | SK<br>(5000 U/mL)             | 37            |             | Yes           |           |     | 170 kHz               | .5 W/cm <sup>2</sup><br>(ISATA)     | c.w.     | 100       | 65            | Measured<br>reperfusion rate                                                                                                                                                                                     | 100%                                                                                                                                                                                                | 22   |  |
|               |                               |               |             |               | Yes       |     |                       |                                     |          |           | 34            |                                                                                                                                                                                                                  | 100%                                                                                                                                                                                                |      |  |
|               |                               |               |             | Yes           |           |     |                       |                                     |          |           | 39            |                                                                                                                                                                                                                  | 100%                                                                                                                                                                                                |      |  |
|               |                               |               |             |               | Yes       |     | 1 MHz                 | 1 W/cm <sup>2</sup><br>(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<br>HIFU | rt-PA<br>(10 µg/mL)           | 37            | Yes         | Yes           |           |     | 1 MHz                 | 60 W                                | 1        | 10        | 30            | Measured volume<br>reduction                                                                                                                                                                                     | 33%                                                                                                                                                                                                 | 23   |  |
|               |                               |               |             |               | Yes       |     | 1 MHz                 | 60 W                                | 1        | 10        | 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<br>(1.0 µg/mL)          | 37            |             | Yes           |           |     | 1 MHz                 | 1, 2, 4,<br>8 W/cm <sup>2</sup>     | c.w.     |           | 60<br>60      | Measured volume<br>reduction                                                                                                                                                                                     | 12.8%<br>18%, 19.3%, 22.8%, 58.7% US at 1 MHz potentiates enzymatic fibrinolysis by a nonthermal mechanism. Thrombolysis efficiency increases with intensity.                                       | 24   |  |
|               |                               | Yes           |             | Yes           | 1 MHz     |     | 2.2 W/cm <sup>2</sup> | n/s                                 | n/s      | 30        | 18%           |                                                                                                                                                                                                                  |                                                                                                                                                                                                     |      |  |
| Human         | UK (200, 2000,<br>5000 µg/mL) | Room          | Yes         |               |           |     | 1 MHz                 | 2.2 W/cm <sup>2</sup>               | n/s      | n/s       | 30            | Measured volume<br>reduction                                                                                                                                                                                     | 18%                                                                                                                                                                                                 | 25   |  |
|               |                               |               |             | Yes           |           |     | 1 MHz                 | 2.2 W/cm <sup>2</sup>               | n/s      | n/s       | 30            |                                                                                                                                                                                                                  | 19%, 44%, 50%                                                                                                                                                                                       |      |  |
|               | SK (50, 250,<br>2000 µg/mL)   |               | Yes         |               | Yes       |     | 1 MHz                 | 2.2 W/cm <sup>2</sup>               | n/s      | n/s       | 30            |                                                                                                                                                                                                                  | 41%, 55%, 61%                                                                                                                                                                                       |      |  |
|               |                               |               |             | Yes           |           |     | 1 MHz                 | 2.2 W/cm <sup>2</sup>               | n/s      | n/s       | 30            |                                                                                                                                                                                                                  | 26%<br>37%, 50%, 62%                                                                                                                                                                                |      |  |
|               |                               |               |             | Yes           |           |     | 1 MHz                 | 2.2 W/cm <sup>2</sup>               | 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<br>(1.0 µg/mL)          | 37            |             | Yes           |           |     | 2.2 MHz               | .5, 1, 2, 4,<br>8 W/cm <sup>2</sup> | n/s      | n/s       | 60<br>60      | Measured volume<br>reduction                                                                                                                                                                                     | 23%<br>31%, 40%, 48%, 69%, 88% US accelerates enzymatic fibrinolysis by increasing transport of reactants through a cavitation-related mechanism. Thrombolysis efficiency increases with intensity. | 26   |  |
|               |                               |               |             |               | Yes       |     |                       |                                     |          |           |               |                                                                                                                                                                                                                  |                                                                                                                                                                                                     |      |  |
| Human         | rt-PA<br>(66.7 µg/mL)         | 37            |             | Yes           |           |     | .5 MHz                | 8 W/cm <sup>2</sup>                 | c.w.     |           | 25<br>25      | Measured volume<br>reduction                                                                                                                                                                                     | 7.24%<br>26.7% US waves accelerate rt-PA-induced thrombolysis and reperfusion.                                                                                                                      | 27   |  |
|               |                               |               |             |               | Yes       |     |                       |                                     |          |           |               |                                                                                                                                                                                                                  |                                                                                                                                                                                                     |      |  |

*(continued on next page)*

**Table 1.** *(continued)*

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

*(continued on next page)*

Table 1. (continued)

| Clot model                           | Drug                  | Temp.<br>(°C) | Mode        |               |            | MBs | Freq.   | Output                                                         | PRF (Hz)       | DF<br>(%)     | Time<br>(min)      | Evaluation<br>method                             | Main conclusion                                                                                                                                                                                                                                                                            | Ref. |
|--------------------------------------|-----------------------|---------------|-------------|---------------|------------|-----|---------|----------------------------------------------------------------|----------------|---------------|--------------------|--------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
|                                      |                       |               | US<br>alone | Drug<br>alone | US + drug  |     |         |                                                                |                |               |                    |                                                  |                                                                                                                                                                                                                                                                                            |      |
| Human                                | rt-PA<br>(3.15 µg/mL) | 33, 37        | Yes         | Yes           | Yes        |     | 120 kHz | 3.2 W/cm <sup>2</sup>                                          | 1667           | 80            | 30                 | Measured volume<br>reduction                     | 7.5%, 7.2%                                                                                                                                                                                                                                                                                 | 34   |
|                                      |                       |               |             |               |            |     |         |                                                                |                |               |                    |                                                  | 8.6%, 12.4%                                                                                                                                                                                                                                                                                |      |
|                                      |                       |               |             |               |            |     | 120 kHz | 3.2 W/cm <sup>2</sup>                                          | 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<br>(10 µg/mL)   | 37            | Yes         | Yes           | Yes        |     | 1.8 MHz | 1.6 MI                                                         | n/s            | n/s           | 60                 | Measured volume<br>reduction                     | 41% 70.8%                                                                                                                                                                                                                                                                                  | 35   |
|                                      |                       |               |             |               |            |     |         |                                                                |                |               | 60                 |                                                  | 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<br>n/s    | Yes<br>Yes  |               |            |     | 220 kHz | 111 W/cm <sup>2</sup>                                          | 2.5            | 50            | .5                 | Measured volume<br>reduction                     | 76.1% (Flow)                                                                                                                                                                                                                                                                               | 36   |
|                                      |                       |               |             |               |            |     | 220 kHz | 111 W/cm <sup>2</sup>                                          | 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<br>(1200 IU)       | 37            |             | Yes<br>Yes    | Yes<br>Yes |     |         |                                                                |                |               | 60                 | Measured volume<br>reduction after<br>incubation | 40.6%                                                                                                                                                                                                                                                                                      | 37   |
|                                      |                       |               |             |               |            |     | 48 kHz  | 5-6 kPa                                                        | n/s            | n/s           | 60                 |                                                  | 59.2%                                                                                                                                                                                                                                                                                      |      |
|                                      |                       |               |             |               |            |     | 225 kHz | 30 mW/cm <sup>2</sup>                                          | n/s            | n/s           | 60, 120<br>60, 120 |                                                  | 8.9%, 46.7%                                                                                                                                                                                                                                                                                |      |
| Bovine through skull flow system     | rt-PA<br>(100 µg/mL)  | 37<br>37      |             | Yes           | Yes<br>Yes |     |         |                                                                |                |               | 29.3<br>17.1       | Measured<br>recanalization rate                  | 100%                                                                                                                                                                                                                                                                                       | 38   |
|                                      |                       |               |             |               |            |     | 1 MHz   | .35 W/cm <sup>2</sup> (I <sub>SPTP</sub> )                     | 16 k           | 41.6          |                    |                                                  | 100%                                                                                                                                                                                                                                                                                       |      |
|                                      |                       |               |             |               |            |     | 185 kHz | 1.27 W/cm <sup>2</sup> (I <sub>SPTP</sub> )                    | 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<br>(100 µg/mL)  | 37            |             | Yes           | Yes        |     | 1 MHz   | .35 W/cm <sup>2</sup> (I <sub>SPTP</sub> )                     | 16,000         | 41.6          | 30<br>30           | Measured<br>recanalization rate                  | 30%<br>90%-100% Transcranial application of 1 MHz US may accelerate reperfusion and recanalization rate of occluded intracerebral vessels.                                                                                                                                                 | 39   |
| Human through skull HIFU flow system |                       | 24            | Yes         |               |            |     | 220 kHz | 13.7, 27.4, 54.8, 136.8 W/cm <sup>2</sup> (I <sub>SPTA</sub> ) | .5, 5, 50, 500 | 5, 10, 20, 50 | .5                 | Measured volume<br>reduction                     | 10.3%-27.2%<br>17.1%-42.9%<br>30%-59.6%<br>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   |

(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%<br>80% The power needed to achieve 80% of thrombolysis with a monofrequency excitation is reduced by half with a bifrequency excitation.                                                   | 41   |
|                                      |                   |            | Yes      |            |           |     | 535 and 565 kHz | 110 W                                               | 3.7      | 10     | 5          |                           |                                                                                                                                                                                                |      |
| Human                                | rt-PA (60 kU/mL)  | 37         |          | Yes        |           |     |                 |                                                     |          |        | 60         | Measured volume reduction | 36.7%<br>40.8%                                                                                                                                                                                 | 42   |
|                                      |                   |            |          |            | Yes       |     | 2 MHz TCCD      | .179 W/cm <sup>2</sup>                              | n/s      | n/s    | 60         |                           |                                                                                                                                                                                                |      |
|                                      |                   |            |          |            | Yes       |     | 2 MHz TCD       | .457 W/cm <sup>2</sup>                              | 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 <sup>2</sup> (I <sub>SPtA</sub> ) | 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 <sub>SPtA</sub> > 35 W/cm <sup>2</sup>            | 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 <sup>2</sup> (I <sub>SAtA</sub> )            | 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 <sup>2</sup> (I <sub>SAtA</sub> )            | 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.    |      |

(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        |           |            |         |                                  |          |        |            |                           |                                                                                                                                |      |
|                                 |                    |            |          |            | Yes       |            | 1.7 MHz | 62.46 W/cm <sup>2</sup> (IsPPA)  | 30       | 8.5    | 10         | Measured volume reduction | 13.6%                                                                                                                          | 49   |
|                                 |                    |            |          |            |           |            | 1.7 MHz | 180.02 W/cm <sup>2</sup> (IsPPA) | 40       | 4      | 10         |                           | 21.4%                                                                                                                          |      |
| Human                           | rt-PA (96 µg/mL)   | 37         | Yes      | Yes        |           | Yes        | 120 kHz | .32 MPa                          | 1667     | 80     | 30         | Measured volume reduction | 46.1% Thrombolysis efficiency increases with intensity.                                                                        | 50   |
|                                 |                    |            |          |            | Yes       |            | 120 kHz | .32 MPa                          | 1667     | 80     | 30         |                           | 6.3%                                                                                                                           |      |
|                                 |                    |            |          |            | Yes       | Yes        | 120 kHz | .32 MPa                          | 1667     | 80     | 30         |                           | 13.0%                                                                                                                          |      |
|                                 |                    |            |          |            | Yes       | Yes        | 120 kHz | .32 MPa                          | 1667     | 80     | 30         |                           | 16.0%                                                                                                                          |      |
| Bovine                          | rt-PA (1000 IU/mL) | 37         | Yes      | Yes        |           | Yes        | 500 kHz | .7 W/cm <sup>2</sup>             | c.w.     | 100    | 1          | Measured volume reduction | 26.2% MBs administration further increases the effect of US on rt-PA induced thrombolysis.                                     | 49   |
|                                 |                    |            |          |            | Yes       |            | 500 kHz | .7 W/cm <sup>2</sup>             | c.w.     | 100    | 1          |                           | 25.8%                                                                                                                          |      |
|                                 |                    |            |          |            | Yes       | Yes        | 500 kHz | .7 W/cm <sup>2</sup>             | c.w.     | 100    | 1          |                           | 24.2%                                                                                                                          |      |
|                                 |                    |            |          |            | Yes       | Yes        | 500 kHz | .7 W/cm <sup>2</sup>             | c.w.     | 100    | 1          |                           | 24.9%                                                                                                                          |      |
| Human Catheter type             | rt-PA (5000 IU/mL) | 37         | Yes      | Yes        |           | Yes        | 1.7 MHz | 4.9 W/cm <sup>2</sup> IsATA      | n/s      | n/s    | 30         | Measured volume reduction | 29.2% MBs have slightly accelerated the thrombolytic effect of rt-PA.                                                          | 51   |
|                                 |                    |            |          |            | Yes       |            | 1.7 MHz | 4.9 W/cm <sup>2</sup> IsATA      | n/s      | n/s    | 30         |                           | .95%                                                                                                                           |      |
|                                 |                    |            |          |            | Yes       |            | 1.7 MHz | 4.9 W/cm <sup>2</sup> IsATA      | n/s      | n/s    | 30         |                           | 7.68%                                                                                                                          |      |
|                                 |                    |            |          |            | Yes       | Yes        | 1.7 MHz | 4.9 W/cm <sup>2</sup> IsATA      | n/s      | n/s    | 30         |                           | 11.10%                                                                                                                         |      |
| Human flow system               | rt-PA (20 µg/mL)   | 37         | Yes      |            |           |            | 2 MHz   | 455 mW/cm <sup>2</sup> IsPTA     | 5000     | n/s    | 30         | Measured volume reduction | 14.41% SC plays an important role in MB-enhanced US accelerated rt-PA-mediated thrombolysis.                                   | 52   |
|                                 |                    |            |          |            |           | Yes        |         | 455 mW/cm <sup>2</sup> IsPTA     | 5000     | n/s    | 30         |                           | 6.1%                                                                                                                           |      |
|                                 |                    |            |          |            | Yes       |            | 2 MHz   | 455 mW/cm <sup>2</sup> IsPTA     | 5000     | n/s    | 30         |                           | 10.9%                                                                                                                          |      |
|                                 |                    |            |          |            | Yes       | Yes        | 2 MHz   | 455 mW/cm <sup>2</sup> IsPTA     | 5000     | n/s    | 30         |                           | 13.1%                                                                                                                          |      |
| Rabbit                          |                    | 25         | Yes      |            |           | Yes (3 µm) | 1 MHz   | .1 W/cm <sup>2</sup>             | 100      | 20     | 30         | Measured volume reduction | 30.7% The application of MBs strongly accelerates lysis of clots exposed to low-intensity US with rt-PA.                       | 53   |
|                                 |                    |            |          |            |           | Yes (1 µm) | 3 MHz   | 2 W/cm <sup>2</sup>              | 100      | 20     | 30         |                           | 18%                                                                                                                            |      |
| Porcine flow system             | rt-PA (7.1 µg/mL)  | 37         |          | Yes        |           |            |         |                                  |          |        |            |                           |                                                                                                                                |      |
|                                 |                    |            |          |            | Yes       | Yes        | 120 kHz | .44 MPa                          | c.w.     | 100    | 30         | Measured volume reduction | 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). | 54   |
|                                 |                    |            |          |            | Yes       | Yes        | 120 kHz | .44 MPa                          | c.w.     | 100    | 30         |                           | 29%                                                                                                                            |      |
|                                 |                    |            |          |            |           |            |         |                                  |          |        |            |                           | 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.<br>(°C) | Mode        |               | US + drug | MBs | Freq.   | Output                                      | PRF (Hz) | DF<br>(%) | Time<br>(min) | Evaluation<br>method                                                                                 | Main conclusion                                                                                                                                                                              | Ref. |
|---------------------------|---------------------------|---------------|-------------|---------------|-----------|-----|---------|---------------------------------------------|----------|-----------|---------------|------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
|                           |                           |               | US<br>alone | Drug<br>alone |           |     |         |                                             |          |           |               |                                                                                                      |                                                                                                                                                                                              |      |
| Human<br>flow<br>system   | rt-PA<br>(3 µg/mL)        | 37            |             | Yes           |           |     |         |                                             |          |           | 60            | Measured clot<br>diameter loss                                                                       | 6.6 µm/min                                                                                                                                                                                   | 55   |
|                           | rt-PA<br>(.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<br>flow<br>system | rt-PA<br>(3 µg/mL)        | 37            |             | Yes           |           |     |         |                                             |          |           | 30            | Measured volume<br>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<br>Flow<br>system | rt-PA<br>(1 µg/mL)        | 37            | Yes         |               |           | Yes | 1 MHz   | 1.5 MPa                                     | .34      | .17       | 20            | Measured lytic<br>efficacy In terms of<br>pressure change<br>(thrombotic<br>occlusion = 40 mm<br>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<br>flow<br>system |                           |               | Yes         |               |           | Yes | 1.6 MHz | .2 (MI)                                     | n/s      | n/s       | 10            | Measured volume<br>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<br>flow<br>system   | rt-PA<br>(.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<br>flow<br>system   | rt-PA<br>(3 µg/mL)        | 37            |             | Yes           |           |     |         |                                             |          |           | 60            | Measured fibrin<br>degradation<br>product (FDP)                                                      | 51.7%                                                                                                                                                                                        | 60   |
|                           |                           |               |             |               | Yes       | Yes | 1 MHz   | 528 mW/cm <sup>2</sup><br>I <sub>SPTA</sub> | .8       | 40        | 60            |                                                                                                      | 53.2% (SC)                                                                                                                                                                                   |      |
|                           |                           |               |             |               | Yes       | Yes | 1 MHz   | 323 mW/cm <sup>2</sup><br>I <sub>SPTA</sub> | .8       | 8         | 60            |                                                                                                      | 57.2% (SC + IC)                                                                                                                                                                              |      |
|                           |                           |               |             |               | Yes       | Yes | 1 MHz   | 3 mW/cm <sup>2</sup><br>I <sub>SPTA</sub>   | .8       | .08       | 60            |                                                                                                      | 50.9% (SC + IC)                                                                                                                                                                              |      |
|                           |                           |               |             |               | Yes       | Yes | 1 MHz   | 45 mW/cm <sup>2</sup><br>I <sub>SPTA</sub>  | .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.<br>(°C) | Mode        |               |           | MBs | Freq.  | Output  | PRF (Hz) | DF<br>(%) | Time<br>(min) | Evaluation<br>method         | Main conclusion                                                                                          | Ref. |
|-------------------------------|----------------------|---------------|-------------|---------------|-----------|-----|--------|---------|----------|-----------|---------------|------------------------------|----------------------------------------------------------------------------------------------------------|------|
|                               |                      |               | US<br>alone | Drug<br>alone | US + drug |     |        |         |          |           |               |                              |                                                                                                          |      |
| Porcine<br>FUS flow<br>system | rt-PA<br>(3.5 µg/mL) | 37            | Yes         | Yes           |           | Yes | 1 MHz  | 20 W    | 100      | 10        | 30            | Measured volume<br>reduction | 31%                                                                                                      | 61   |
|                               |                      |               |             |               |           |     |        |         |          |           | 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<br>flow<br>system       | rt-PA<br>(7 µg/mL)   | 37            | Yes         | Yes           |           |     | .6 MHz | 60 W    | 100      | 10        | 30            | Measured volume<br>reduction | 29.2%                                                                                                    | 62   |
|                               |                      |               |             |               | Yes       |     | 1 MHz  | 20-60 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%                                                                                                    |      |

Abbreviations: Abib, abciximab immunobubbles; c.w., continuous wave; DF, duty factor; ELIP, echogenic liposomes; Eptf, eptifibatide; Freq., frequency; FUS, focused ultrasound; HIFU, high-intensity focused ultrasound; IA, intrarterial; IV, intravenous; MBs, microbubbles; MCA, middle cerebral artery; mt-PA, alteplase 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        |           |     |          |                        |              |        |            |                                            |                                                                                                                                                                                                                                   |              | 1    |
|                                      |                  |          |            | Yes       |     | 1 MHz    | 1.75 W/cm <sup>2</sup> | Intermittent | 50     | 50, 200    | Measured volume reduction                  | 2%0, 30%<br>41%, 55%                                                                                                                                                                                                              |              |      |
|                                      | rt-PA (2 mg)     |          | Yes        | Yes       |     | 1 MHz    | 1.75 W/cm <sup>2</sup> | Intermittent | 50     | 50, 200    |                                            | 28%, 40%<br>45%, 50%                                                                                                                                                                                                              |              |      |
| Rat MCA stroke                       | rt-PA (10 mg/kg) |          | Yes        |           |     |          |                        |              |        | 60         | Measured relative infarct volume reduction | 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. | 18% ICH      | 63   |
|                                      | rt-PA (5 mg/kg)  |          |            | Yes       |     | 25.6 kHz | .6 W/cm <sup>2</sup>   | n/s          | 20     | 60         |                                            | 51%                                                                                                                                                                                                                               |              |      |
|                                      | rt-PA (10 mg/kg) |          |            | Yes       |     | 25.6 kHz | .6 W/cm <sup>2</sup>   | n/s          | 20     | 60         |                                            | 68%<br>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.                             | 20% ICH      |      |
| 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      |      |
| Rabbit MCA Stroke HIFU               |                  | Yes      |            |           | Yes | 1.5 MHz  | 88-137 W               | 1            | .1     | .33        | Measured recanalization rate               | HIFU, as a stand-alone method, can cause effective thrombolysis and does not damage the targeted vessels.                                                                                                                         | 22% ICH      | 65   |
|                                      |                  | Yes      |            |           | Yes | 1.5 MHz  | 88 W                   | 10           | .1     | .33        |                                            | 78% (7/9)<br>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)<br>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.           | 13% ICH      |      |

(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)                                                                                                                                                                                                  |              |      |
|                                         |                                                                  |          |            |           |            |         |                     |          |        |            |                                                       | 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 <sup>2</sup> | 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      |         |                     |          |        | 2          |                                                       | 60%                                                                                                                                                                                                           |              |      |
|                                         |                                                                  |          |            | Yes       | ELIP       | 5.7 MHz | 1.25 MPa            | 5 k      | n/s    | 2          |                                                       | 100%                                                                                                                                                                                                          |              |      |
|                                         |                                                                  |          |            |           | rt-PA      |         |                     |          |        |            |                                                       | 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<br>MCA stroke                     | rt-PA<br>(1.2 mg/<br>animal)                                                              |          | Yes        |           |     |         |                                                |          |        | 32         | Measured volume<br>reduction    | 45% (9/20)                                                                                                                                                                                       |              | 7    |
|                                       |                                                                                           |          |            | Yes       |     | 490 kHz | .8 W/cm <sup>2</sup>                           | c.w.     | 100    | 32         |                                 | 76.2% (16/21)<br>Low-frequency transcranial US under appropriate conditions could be an effective and safe method of treatment for ischemic stroke.                                              |              |      |
| Rabbit<br>Femoral artery<br>occlusion | mt-PA<br>(1.2 mg/<br>animal)                                                              |          | Yes        |           |     |         |                                                |          |        | 32         | Measured<br>recanalization rate | 16.7% (2/12)                                                                                                                                                                                     |              | 69   |
|                                       |                                                                                           |          |            | Yes       |     | 490 kHz | .13 W/cm <sup>2</sup>                          | c.w.     | 100    | 32         |                                 | 66.7% (6/9)<br>Low-frequency and low-intensity transcranial US enhanced thrombolysis by mt-PA.                                                                                                   |              |      |
| Rabbit<br>Femoral artery<br>occlusion | SK<br>(15,000 U/<br>kg) as bolus<br>followed by<br>an infusion<br>of<br>15,000 U/<br>kg/h | Yes      | Yes        |           |     | 40 kHz  | .75 W/cm <sup>2</sup>                          | c.w.     | 100    | 120        | Measured<br>reperfusion rate    | <7%                                                                                                                                                                                              |              | 70   |
|                                       |                                                                                           |          |            | Yes       |     | 40 kHz  | .75 W/cm <sup>2</sup>                          | c.w.     | 100    | 120        |                                 | 7%                                                                                                                                                                                               |              |      |
|                                       |                                                                                           |          |            |           |     |         |                                                |          |        |            |                                 | 83%<br>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<br>MCA stroke<br>HIFU          | rt-PA (1.mg/<br>ml/kg)                                                                    |          | Yes        |           |     |         |                                                |          |        | 120        | Measured<br>recanalization rate | 100%                                                                                                                                                                                             |              | 71   |
|                                       |                                                                                           |          |            | Yes       | Yes | 1 MHz   | 20 W/cm <sup>2</sup><br>(I <sub>SATA</sub> )   | 10       | 10     | 70         |                                 | 100%<br>HIFU in combination with rt-PA dissolved clots.                                                                                                                                          |              |      |
| Rabbit<br>MCA stroke<br>HIFU          | rt-PA (1.mg/<br>ml/kg)                                                                    |          |            | Yes       | Yes | 1 MHz   | 20 W/cm <sup>2</sup><br>(I <sub>SATA</sub> )   | 10       | 10     | 70         | Measured<br>recanalization rate | 100%<br>Therapeutic US in synergy with rt-PA dissolve clots.                                                                                                                                     |              | 72   |
| Rabbit<br>Hindlimb<br>occlusion       |                                                                                           | Yes      |            |           | Yes | 1 MHz   | .031 W/cm <sup>2</sup><br>(I <sub>SATA</sub> ) | .33      | .17    | 10         | Measured<br>recanalization rate | 67%                                                                                                                                                                                              |              | 73   |
|                                       |                                                                                           | Yes      |            |           | Yes | 1 MHz   | .031 W/cm <sup>2</sup><br>(I <sub>SATA</sub> ) | .33      | .17    | 20         |                                 | 100%<br>Long-pulse-length US with MBs has a therapeutic effect on microvascular perfusion.                                                                                                       |              |      |
| Rabbit<br>Femoral artery<br>occlusion | rt-PA<br>(30 µg/kg/<br>min)                                                               |          | Yes        |           |     |         |                                                |          |        | 74         | Measured initial<br>reflow      | 15%-50%                                                                                                                                                                                          |              | 74   |
|                                       |                                                                                           |          |            | Yes       |     | 1 MHz   | 6.3 W/cm <sup>2</sup><br>I <sub>SPTA</sub>     | c.w.     | 100    | 33         |                                 | 15%-50%<br>Although time to initial reflow was shortened by US, it was associated with less reperfusion and more reocclusion in this model.                                                      |              |      |

*(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      | 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%                                                                                                                                                                                                                         |              |      |
|                              |                      |          |            |           |            |                              |                              |          | 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 <sup>2</sup> (IsATA) | 100      | 20     | 60         | Measured infarct volume                | 1%                                                                                                                                                                                                                          | 56% ICH      | 77   |
|                              |                      |          |            | Yes       |            | 1 MHz                        | .8 W/cm <sup>2</sup> (IsATA) | 100      | 20     | 60         |                                        | .13%                                                                                                                                                                                                                        | 61% ICH      |      |
|                              |                      | Yes      |            |           | Yes        | 1 MHz                        | .8 W/cm <sup>2</sup> (IsATA) | 100      | 20     | 60         |                                        | .2%                                                                                                                                                                                                                         | 19% ICH      |      |
|                              |                      |          | Yes        | Yes       | 1 MHz      | .8 W/cm <sup>2</sup> (IsATA) | 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        |           |            | 1 MHz                        | .8 W/cm <sup>2</sup>         | n/s      | 20     | 60         | Measured infarct volume                | 2.2%                                                                                                                                                                                                                        | 45% ICH      | 78   |
|                              |                      | Yes      |            |           | Yes        | 1 MHz                        | .8 W/cm <sup>2</sup>         | n/s      | 20     | 60         |                                        | 1.7%                                                                                                                                                                                                                        | 50% ICH      |      |
|                              |                      |          |            |           |            |                              |                              |          |        |            |                                        | Sonothrombolysis without rt-PA using MBs is effective in decreasing infarct volumes.                                                                                                                                        | 36% ICH      |      |

*(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      | Yes        |           |       | 1 MHz  | .8 W/cm <sup>2</sup>  | n/s      | 20                                                                                                                   | 60         | Measured infarct volume | .97%            | 56% ICH      | 79   |              |
|                                                                                                                                          |                   |          |            |           |       |        |                       |          |                                                                                                                      | 60         |                         | .14%            | 48% ICH      |      |              |
|                                                                                                                                          |                   |          |            | Yes       |       | 1 MHz  | .8 W/cm <sup>2</sup>  | n/s      | 20                                                                                                                   | 60         |                         | .15%            | 73% ICH      |      |              |
|                                                                                                                                          |                   | Yes      |            |           | Yes   | 1 MHz  | .8 W/cm <sup>2</sup>  | n/s      | 20                                                                                                                   | 60         |                         | .20%            | 19% ICH      |      |              |
|                                                                                                                                          |                   |          |            | Yes       | Yes   | 1 MHz  | .8 W/cm <sup>2</sup>  | 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      | Yes        |           |       | 20 kHz | 1.5 W/cm <sup>2</sup> | n/s      | n/s                                                                                                                  | 60         | Measured patency rate   | 0% (0/6)        |              | 80   |              |
|                                                                                                                                          |                   |          |            |           |       |        |                       |          | 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 <sup>2</sup>                                                                                                | n/s        |                         | n/s             | 60           |      | 87% (13/15)  |
|                                                                                                                                          |                   | Yes      |            |           | Yes   | 20 kHz | 1.5 W/cm <sup>2</sup> | 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<br>MCA occlusion | rt-PA<br>(.9 mg/kg) |            |            | Yes (TCD)  | Yes | 2 MHz   | n/s                            | n/s      | n/s    | 120        | Measured complete and partial recanalization rate | 50% (6/12)<br>33% (4/12)<br>MBs reached and permeated beyond occlusions with no increase in sICH suggesting the feasibility of further studies                                                | 0% sICH<br>0% sICH                  | <a href="#">81</a> |
| Stroke patients<br>MCA occlusion |                     | Yes (TCD)  |            |            |     | 2 MHz   | n/s                            | n/s      | n/s    | 60         | Measured complete recanalization rate             | 30% (4/12)<br>Sonothrombolysis using 2 probes and bilateral monitoring is safe but not more effective than standard sonothrombolysis.                                                         | 0% sICH                             | <a href="#">82</a> |
| Stroke patients<br>MCA occlusion |                     | Yes (TCCD) |            |            |     | 2 MHz   | 415 mW/cm <sup>2</sup> (ISPTA) | n/s      | n/s    | 30         | Measured partial recanalization rate              | 83% (5/6)<br>High rate of early partial recanalization during continuous exposure to 2-MHz US without rt-PA.                                                                                  | 0% sICH                             | <a href="#">83</a> |
| Stroke patients<br>MCA occlusion | rt-PA<br>(.9 mg/kg) |            | Yes        |            |     |         |                                |          |        | 120        | Measured complete recanalization rate             | 24% (9/36)<br>41% (15/37)<br>55% (21/38)<br>MBs administration induces further acceleration of US-enhanced thrombolysis.                                                                      | 5.5% sICH<br>2.7% sICH<br>2.6% sICH | <a href="#">84</a> |
|                                  |                     |            |            | Yes (TCD)  | Yes | 2 MHz   | n/s                            | n/s      | n/s    | 120        |                                                   |                                                                                                                                                                                               |                                     |                    |
|                                  |                     |            |            | Yes (TCD)  | Yes | 2 MHz   | n/s                            | n/s      | n/s    | 120        |                                                   |                                                                                                                                                                                               |                                     |                    |
| Stroke patients<br>MCA occlusion | rt-PA<br>(.9 mg/kg) |            |            | Yes (TCCD) |     | 2 MHz   | 189 mW/cm <sup>2</sup>         | n/s      | n/s    | 60         | Measured complete recanalization rate             | 53% (8/15)<br>64% (7/11)<br>MBs enhanced TCCD monitored rt-PA thrombolysis lead to a greater immediate clinical improvement.                                                                  | 7% sICH<br>9% sICH                  | <a href="#">85</a> |
|                                  |                     |            |            | Yes (TCCD) | Yes | 2 MHz   | 189 mW/cm <sup>2</sup>         | n/s      | n/s    | 60         |                                                   |                                                                                                                                                                                               |                                     |                    |
| Stroke patients<br>MCA occlusion | rt-PA<br>(.9 mg/kg) |            | Yes        |            |     |         |                                |          |        | 60         | Measured complete and partial recanalization rate | 21.4% (3/14—complete)<br>0% (0/14—partial)<br>27.3% (3/11—complete)<br>18.2% (2/11—artial)<br>Transcranial TCCD with rt-PA showed a higher grade of recanalization compared with rt-PA alone. | 7% sICH                             | <a href="#">8</a>  |
|                                  |                     |            |            | Yes (TCCD) |     | 2-4 MHz | 179 mW/cm <sup>2</sup>         | n/s      | n/s    | 60         |                                                   |                                                                                                                                                                                               | 36% sICH                            |                    |
| Stroke patients<br>MCA occlusion | rt-PA<br>(.9 mg/kg) |            | Yes        |            |     |         |                                |          |        | 120        | Measured complete recanalization rate             | 30% (19/63)                                                                                                                                                                                   | 5% sICH                             | <a href="#">9</a>  |
|                                  |                     |            |            | Yes (TCD)  |     | 2 MHz   | 750 mW/cm <sup>2</sup>         | n/s      | n/s    | 120        |                                                   | 49% (31/63)<br>Continuous TCD augments rt-PA-induced arterial recanalization.                                                                                                                 | 5% sICH                             |                    |
| Stroke patients<br>MCA occlusion | rt-PA<br>(.9 mg/kg) |            |            | Yes (TCD)  |     | 2 MHz   | n/s                            | n/s      | n/s    | 120        | Measured complete recanalization rate             | 36% (20/55)<br>Complete recanalization within 2 h after rt-PA bolus is a feasible goal for thrombolysis given with TCD monitoring.                                                            | 6% sICH                             | <a href="#">86</a> |

(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<br>MCA occlusion                   | rt-PA<br>(.9 mg/kg)                     |            | Yes        |            |              |         |                                |          |        | 60         | Measured complete and partial recanalization rate        | 11.1% (2/18—complete)<br>11.1% (2/18—partial)                                                                                                                                                                                                                                            | 5.6% sICH              | 10   |
|                                                    |                                         |            |            | Yes (TCCD) |              | 1.8 MHz | 179 mW/cm <sup>2</sup>         | n/s      | n/s    | 60         |                                                          | 15.8% (3/19—complete)<br>42.1% (8/19—partial)<br>Transcranial US in combination with rt-PA accelerates recanalization in MCA occlusion, compared with rt-PA alone                                                                                                                        | 15.8% sICH             |      |
| Stroke patients<br>MCA occlusion                   | rt-PA<br>(.9 mg/kg)                     |            | Yes        |            |              |         |                                |          |        | 90         | Measured recanalization rate (both complete and partial) | 50% (6/12)<br>29% (4/14)                                                                                                                                                                                                                                                                 | 0% sICH                | 87   |
|                                                    |                                         |            |            | Yes        |              | 300 kHz | 700 mW/cm <sup>2</sup> (IsPTA) | 100      | 5      | 90         |                                                          | Low frequency US combined with rt-PA showed an increased rate of sICH.                                                                                                                                                                                                                   | 36% sICH               |      |
| Stroke patients<br>Proximal intracranial occlusion | rt-PA<br>(.9 mg/kg)                     |            | Yes        |            |              |         |                                |          |        | 90         | Measured complete and partial recanalization rate        | 33% (4/12—complete)<br>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)<br>17% (2/12—partial)                                                                                                                                                                                                                                                | 0% sICH                |      |
|                                                    |                                         |            |            | Yes (TCD)  | Yes (2.8 mL) | 2 MHz   | n/s                            | n/s      | 100    | 90         |                                                          | 45% (5/11—complete)<br>0% (0/11—partial)<br>MBs can be safely combined with systemic rt-PA and US at a dose of 1.4 mL.                                                                                                                                                                   | 27% sICH               |      |
| Stroke patients<br>MCA occlusion                   | rt-PA<br>(.9 mg/kg)                     | Yes (TCCD) |            |            |              |         |                                |          |        | 45         | Measured complete and partial recanalization rate        | 97% (36/37—complete)<br>0% (0/37—partial)                                                                                                                                                                                                                                                | 2.7% sICH              | 89   |
|                                                    |                                         |            | Yes        |            |              |         |                                |          |        | 45         |                                                          | 67% (10/15—complete)<br>7% (1/15—partial)                                                                                                                                                                                                                                                | 6.6% sICH              |      |
|                                                    |                                         |            |            | Yes (TCCD) |              | 2-4 MHz | 208 W/cm <sup>2</sup> (IsPPA)  | 5 k      | .7     | 45         |                                                          | 80% (12/15—complete)<br>7% (1/15—partial)<br>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<br>MCA occlusion<br>Catheter type  | rt-PA<br>(60 mg IV + 22 mg IA infusion) |            | Yes        | Yes        |              | 1.7 MHz | n/s                            | n/s      | n/s    | 120<br>120 | Measured recanalization rate (both complete and partial) | 56% (33/59)<br>73% (24/33)<br>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<br>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,<sup>8,84,85,88</sup> 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,<sup>19</sup> causes additional binding sites due to reversible disaggregation of fibrin fibers,<sup>18</sup> and increases the binding of thrombolytic agents to fibrin.<sup>17</sup>

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.<sup>34</sup> 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.<sup>25,59</sup> 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.<sup>23,30,35</sup> 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<sup>43,44,46</sup> 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,<sup>23,30,35</sup> 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.<sup>11,29</sup> 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.<sup>16,37,38</sup> Considering that some other studies showed that sonothrombolysis efficacy increases as the level of acoustic intensity increases,<sup>24,26,28</sup> 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,<sup>50</sup> animal,<sup>75</sup> and clinical<sup>84</sup> 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.<sup>48,51,54</sup> However, a study performed by Molina et al<sup>88</sup> 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,<sup>87</sup> 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<sup>63</sup> and the level of acoustic intensity.<sup>64,65</sup>

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<sup>1,33</sup> 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<sup>2</sup>) diagnostic transcranial US in combination with rt-PA.<sup>83,85</sup> Using this protocol, higher recanalization rates were exhibited compared with those with rt-PA alone.

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