Review Article

Review of Protocols Used in Ultrasound Thrombolysis

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

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

Introduction

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

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

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

Received October 8, 2016; revision received July 1, 2017; accepted July 30, 2017.

Address correspondence to Christakis Damianou, PhD, Electrical Engineering Department, Cyprus University of Technology, Cyprus. E-mail: christakis.damianou@cut.ac.cy.

1052-3057/$ - see front matter

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

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

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

Materials and Methods

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

Results

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

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

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

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