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Stability of alteplase in presence of cavitation

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Abstract

Several experimental studies have demonstrated that ultrasound (US) can accelerate enzymatic fibrinolysis and this effect is further enhanced in the presence of ultrasound contrast agents (UCA). Although UCA have been shown to be safe when administered to ischemic stroke patients, safety information of these agents in the thrombolysis setting is limited. Therefore, in this study we investigated potential adverse effects of acoustic cavitation generated by UCA on alteplase (t-PA), the drug used for treatment of ischemic stroke patients. A volume of 0.9 mL of alteplase was dispensed into a custom-made polyester sample tube. For treatments in the presence or absence of cavitation either 0.1 mL Optison or phosphate buffer saline was combined with alteplase. Three independent samples of each treatment group were exposed to ultrasound of 2 MHz frequency at three different peak negative acoustic pressures of 0.5, 1.7, and 3.5 MPa for a duration of 60 min. All treatments were carried out in a cavitation detection system which was used to insonify the samples and record acoustic emissions generated within the sample. After ultrasound exposure, the treated samples and three untreated drug samples were tested for their enzymatic activity using a chromogenic substrate. The insonified samples containing Optison demonstrated cavitational activity proportional to acoustic pressure. No significant cavitation activity was observed in the absence of Optison. Enzymatic activity of alteplase in both insonified groups was comparable to that in the control group. These tests demonstrated that exposure of alteplase to 60 min of 2 MHz ultrasound at acoustic pressures ranging from 0.5 MPa to 3.5 MPa, in the presence or absence of Optison had no adverse effects on the stability of this therapeutic compound.

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Keywords: Acoustic cavitation; Sonothrombolysis; Enzymatic stability; Sonochemistry; Alteplase

1. Introduction

It is well known that ultrasound (US) can accelerate clot dissolution by plasminogen activators (PA) [1–3]. Although the mechanisms are not fully understood, it is speculated that ultrasound accelerates enzyme mediated thrombolysis primarily through mechanical effects, by increasing transport of drug molecules into the clot [4–6]. In particular, cavitation has been identified as a mechanism that can significantly enhance this process [7]. The addition of ultrasound contrast agents (UCA), which act as cavitation nuclei, has been shown to increase the effectiveness of ultrasound-accelerated thrombolysis [8–11]. The feasibility of this approach has been demonstrated in the treatment of ischemic stroke by Molina et al. [12] and Viguier et al. [13]. Molina et al. reported that administration of UCA induces further acceleration of US-enhanced thrombolysis in acute ischemic stroke leading to a more complete recanalization. Although UCA have been shown to be safe when administered to acute ischemic stroke patients [14,15], there is limited information available as to the impact of UCA on alteplase stability in the presence of ultrasound at the acoustic pressure levels relevant to ischemic stroke treatment. Smikahl et al. [16] demonstrated that alteplase

Abbreviations: US, Ultrasound; ANOVA, ANalysis Of VAriance; OP, Optison; UCA, Ultrasound contrast agent; PA, Plasminogen activator; TCD, Transcranial Doppler.

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exposed to a 20 kHz vibrating wire for 6 min remained fully active and stable. Although the presence of cavitation was not directly verified during the tests reported by Smikahl et al., it was proposed as the principle mechanism for this device [17].

In a previous study we demonstrated that ultrasound (1 MHz, 2.5–3.1 W/cm^2) did not affect the biological activity of four plasminogen activators, including alteplase [18]. Meanwhile, we learned that the acoustic output used in the previous study was not high enough to generate cavitation in a clean, particulate-free, solution. Considering that cavitation is known to be mechanism responsible for causing biochemical reactions in sonochemistry [19] by causing bond dissociations in molecules and produce free radicals that can react with biomolecular materials [20–22], we designed the current study to investigate the impact of acoustic cavitation arising from UCAs on the stability of alteplase. Any adverse effect caused by ultrasound to alteplase that would lead to inactivation, denaturation, or fragmentation due to high temperature, microjets, free radical generation, acoustic streaming and increased shear stress, will lead to a decrease in enzymatic activity of this enzyme which could be detected using a well established chromogenic assay [1,23–28]. Enzymatic activity was evaluated after 60 min of exposure to 2 MHz ultrasound at various acoustic pressure amplitudes in the presence and absence of a UCA called Optison while the type (stable, inertial), relative quantity and duration of cavitation was acoustically monitored in real-time.

2. Materials and methods

2.1. Sample preparation

Alteplase (Activase®; Genentech Inc., South San Francisco, CA) was reconstituted to a concentration of 580,000 IU/mL. 0.9 mL of the alteplase was dispensed into a custom-made, 9.4 mm ID, thin-walled (0.0020″ wall), polyester test tube chosen for its acoustic transparency. For treatments in which cavitation was desired, 0.1 mL Optison (Amersham Health Inc., Princeton, NJ, USA) was added to the alteplase to give an Optison volume concentration of 10% v/v, which is approximately 50 times greater than the specified maximum total dose for intravenous application of Optison, which is 0.2% v/v or 8.7 mL in ~4.7 L blood. For treatments without contrast agent, 0.1 mL phosphate buffered saline was combined with the alteplase. In all cases, the final alteplase concentration was 522,000 IU/mL. The reason for choosing higher concentrations of alteplase and Optison than used in a clinical setting was to increase the measurement sensitivity. A high Optison concentration will enhance the cavitational activity [29] and a high concentration of alteplase molecules will increase the molecular interaction [30].

2.2. Treatment protocols

Three main treatment protocols were tested: (1) A—alteplase-only control; (2) A + US—alteplase exposed to ultrasound in the absence of Optison; and (3) A + US + OP—alteplase exposed to ultrasound in the presence of Optison. For both protocols with ultrasound, three different acoustic pressure levels were investigated (resulting in a total of seven treatment protocols). Three independent samples were tested for each of the treatment protocols [25,26].

2.3. Experimental setup

All treatments were carried out in a cavitation detection system, which was used to induce cavitation, and record scattered acoustic emissions generated within the sample. Fig. 1 shows a diagram of the experimental setup. The water bath was heated to 37 °C, filtered to 0.2 μm, and degassed to less than 36% of saturation. The test tube containing alteplase sample was lowered into the water bath where it remained for the duration of the 60 min treatment. A magnetic, stir bar (5 mm length) at the bottom of the sample tube, controlled by a magnetic stir plate positioned 4 cm beneath the sample tube base, rotated at 400 rpm to

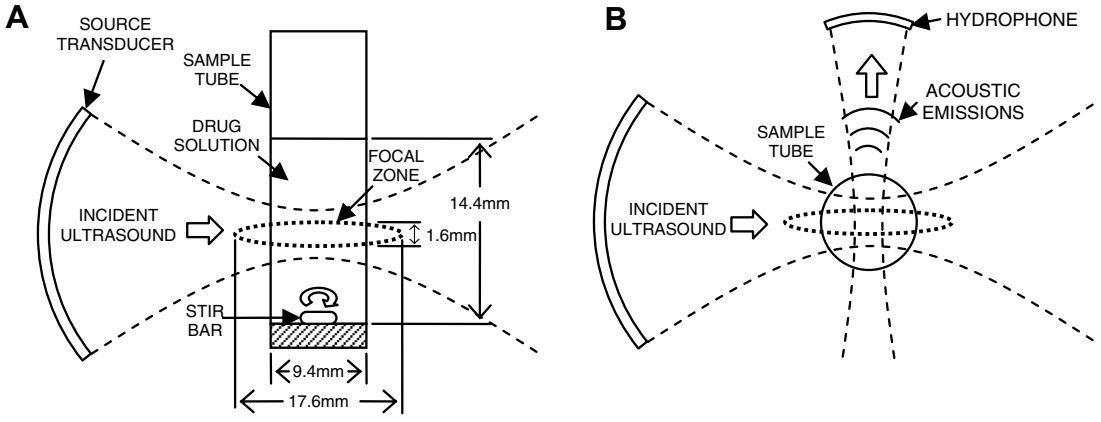

Fig. 1. Diagram of experimental setup: (A) side view; (B) top view.