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Dependence of pulsed focused ultrasound induced thrombolysis on duty cycle and cavitation bubble size distribution

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ABSTRACT

In this study, we investigated the relationship between the efficiency of pulsed, focused ultrasound (FUS)-induced thrombolysis, the duty cycle (2.3%, 9%, and 18%) and the size distribution of cavitation bubbles. The efficiency of thrombolysis was evaluated through the degree of mechanical fragmentation, namely the number, mass, and size of clot debris particles. First, we found that the total number and mass of clot debris particles were highest when a duty cycle of 9% was used and that the mean diameter of clot debris particles was smallest. Second, we found that the size distribution of cavitation bubbles was mainly centered around the linear resonance radius (2.5 μm) of the emission frequency (1.2 MHz) of the FUS transducer when a 9% duty cycle was used, while the majority of cavitation bubbles became smaller or larger than the linear resonance radius when a 2.3% or 18% duty cycle was used. In addition, the inertial cavitation dose from the treatment performed at 9% duty cycle was much higher than the dose obtained with the other two duty cycles. The data presented here suggest that there is an optimal duty cycle at which the thrombolysis efficiency and cavitation activity are strongest. They further indicate that using a pulsed FUS may help control the size distribution of cavitation nuclei within an active size range, which we found to be near the linear resonance radius of the emission frequency of the FUS transducer.

1. Introduction

Thrombosis-related diseases such as myocardial infarction, stroke, pulmonary embolism, deep venous thrombosis, systemic embolism, and others are by far the leading causes of mortality and morbidity worldwide. Therefore, various thrombolytic therapies have been developed, and some have progressed to clinical trials and shown effective results [1]. Current thrombolytic therapies used in the clinic include thrombolytic drugs [2,3], catheter-based intravascular techniques [4,5], or a combination of the two [6,7]. Each of these methods has its own set of drawbacks. Drugs are non-site-specific, resulting long treatment times (several hours) and are associated with risk of bleeding. Catheters also carry a risk of bleeding and are invasive to the blood vessel wall.

The potential of using ultrasound (US) in the treatment of thrombolytic diseases has been extensively studied for several decades. US is a completely novel, non-pharmacological approach that offers the potential to increase reperfusion and limit bleeding complications, either alone or in combination with US contrast agents. US can also be used as an adjunct to thrombolytic therapy [8–10]. However, treatments in which US are used in combination with thrombolytic drugs can still cause drug-associated hemorrhaging. In addition, treatments in which US was used in combination with US contrast agents risk damage to blood vessels due to the uncontrollable rupturing of US contrast agents [11,12]. Catheter-delivered external transducer US is a technique that has been researched by many groups. However, it is considerably more invasive than externally-delivered US and suffers from high technical demands and other potential risks.

Focused US (FUS) has been shown to be an effective thrombolytic method without thrombolytic drugs both in vitro and in vivo. It is non-invasive and requires only short treatment times (several minutes) [13–15]. Although the feasibility of using FUS to induce thrombolysis has been determined, there is still the clinical concern that any clot debris produced by the fragmentation of the original clot may block microvessels in distal vascular beds. Therefore, it is essential to measure the size distribution of clot debris following FUS-mediated lysis and to evaluate how the size distribution of the clot debris may be related to the acoustic parameters of FUS so that the FUS-induced thrombolytic effect may be optimized. Current research on FUS-induced thrombolysis has been focused on the feasibility of using this technique to break

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clots [13–15], while there have only been very limited studies on the size distribution of the resulting clot debris.

In papers where high-intensity FUS was used to break down blood clots, cavitation collapse was suggested to be the underlying cause of clot lysis [8,13]. The collapse of bubbles in a fluid subjected to US gives rise to transient, yet powerful microjets that can disintegrate clots mechanically. Previous studies have shown that thrombolysis is more efficient using pulsed FUS rather than continuous FUS [13], as the degree of acoustic shielding is minimized by the relatively long pauses between pulses, allowing intense cavitation to take place in the focus [8,13]. More specifically, our hypothesis on the mechanism of enhanced thrombolysis using pulsed US is that each US pulse creates a cluster of microbubbles localized at the transducer focus. Collapse of the microbubbles causes local stress that removes a portion of the targeted clot. Residual microbubbles may also act as nuclei that can be excited by subsequent pulses, predisposing clot in the focal region to further damage. Other studies have explored how changing acoustic parameters (particularly PRF) can enhance cavitation effects when using pulsed US [16–18]. Therefore, it may be possible to achieve a synergistic effect between residual cavitation nuclei by adjusting the timing between two consecutive pulses. It can be hypothesized then that in pulsed FUS-induced thrombolysis, if the size distribution of microbubbles is within an active size range in which the bubbles oscillate strongly in response to the applied acoustic pressure [19,20], the cavitation activity will be enhanced and result in optimal thrombolysis. To test this hypothesis, we must know the size of microbubbles generated by the treatment process and how they relate to changes in FUS acoustic parameters.

Although pulse amplitude, pulse duration (PD), and pulse frequency have all been reported to affect the size distribution of cavitation bubbles [20–22], pulse frequency cannot be easily changed due to experimental limitations, while pulse amplitude only has a small effect on the distribution of bubble sizes for the range of intensity levels used in US-induced thrombolysis [21,23]. Therefore, we chose to vary the PD by changing duty cycles for a given pulse repetition frequency (PRF) in this study.

Using a fixed PRF, we measured the size distribution of clot debris generated by pulsed FUS to investigate the effect of changing the duty cycle on the efficiency of thrombolysis. The efficiency was evaluated by the degree of mechanical fragmentation of a clot, namely the number, mass, and size of clot debris particles. Furthermore, the evolution of the size distribution of cavitation bubbles in the treatment was estimated to evaluate the relationship between the size distribution and the duty cycle. In addition, cavitation activity during the treatment was monitored using passive cavitation detection (PCD) to compare the difference in cavitation dose between treatments.

2. Methods and materials

2.1. Experimental setup

A schematic representation of the experimental setup is shown in Fig. 1a, including US generation, clot sample, PCD, and cavitation bubble size estimation. Exposures were performed in degassed water maintained at 37 °C within a tank (60 cm × 40 cm × 26 cm) made of optically transparent polycarbonate.

2.2. Ultrasound generation

A single spherical concave FUS transducer (Imasonic, Besancon, France) made from 1 to 3 piezocomposite material was driven by an arbitrary wave generator (AWG 420, Tectronix, Beaverton, OR, USA) and a power amplifier (AG1016, T&G Power Conversion Inc., Rochester, NY, USA). The transducer operated at a center frequency of approximately 1.2 MHz, at which the transmitted efficiency (acoustic power/electrical load power) was 72%. The transducer has a 120 mm radius of curvature, 150 mm aperture, 120 mm focal length, and an f-number of 0.8. The FUS transducer output was calibrated in degassed water with a needle hydrophone (0.5 mm diameter, Precision Acoustics Ltd., Dorchester, UK) connected to a multi-scan system (Panametrics Inc., Waltham, MA, USA) at an acoustic power of 2 W. The full-width half-maximum (FWHM) beam length and width of the transducer were 8 mm and 1.6 mm, respectively [24]. The FUS transducer was pulsed at an acoustic power of 60 W at a PRF of 454 Hz throughout this study (Fig. 2a). Three PDs (50 μs, 200 μs, and 400 μs) per pulse period (1/454 Hz ≈ 2.2 ms) were evaluated, corresponding to three duty cycles (2.3%, 9%, and 18%). The treatment time for each trial lasted 210 s.

2.3. Clot preparation

Fresh non-heparinized blood was obtained from domestic swine from a local slaughter house and placed in sterile vitreous containers. The blood was coagulated at room temperature for 3 h and maintained at 4 °C for up to 3 days to allow for maximal clot retraction, lytic resistance, and stability. The clot was cut using a standard mould into small pieces (approximately 5 mm × 5 mm × 5 mm). Each clot was placed on a 8 μm resinic syringe-top filter and flushed with saline three times to eliminate any fluid generated during cutting. The clot was then carefully placed in a condom filled with saline and fixed on a sample holder. The sample holder was positioned by a computer-controlled three-dimensional positioning system (Suruga Seiki Ltd., Tokyo, Japan).

2.4. Cavitation detection system and data processing

The cavitation detection system consisted of a 5 MHz single-element transducer (band width of 2.45 to 7 MHz, V309, Panametrics, Waltham, MA, USA) and a broadband receiver (BR-640, RETIC, Inc. Warwick, RI, USA). The single-element transducer was fixed above the clot and kept at 45° relative to horizontal. The emitted acoustic signal 30 s before and during the treatment time around the clot were collected by a high-speed digitizer (CS12400, Gage Applied, Inc. Lachine, QC, Canada) with a resolution of 20 ns. The digitizer was set on auto-save mode and automatically captured an 8.8 ms long (550,000 points) waveform once per second (Fig. 2a).

Confocusing of the FUS and the 5 MHz "listening" transducer was performed with a needle hydrophone. First, the spatial position of the focus of the FUS transducer was determined using the needle hydrophone (0.5 mm diameter, Precision Acoustics Ltd., Dorchester, UK). Then, the 5 MHz "listening" transducer was adjusted until the received signal amplitude from the tip of the needle hydrophone was at its maximum.

The recorded passively-detected waveforms were stored for signal processing using MATLAB (MathWorks, Inc., Natick, WA, USA). Each recorded waveform was converted to the frequency domain using fast Fourier transform (FFT). The passively-detected signal in the frequency domain was expressed on a logarithmic scale. The cavitation noise power (CNP) value for each recorded waveform was calculated by integrating the cavitation spectrum in the logarithmic scale across the entire band [25]. By registering each CNP value for a recorded waveform to the time trace, the inertial cavitation dose (ICD) was calculated as the integrated area under the time-CNP value curve over the entire duration of recording.

CNP calculation was performed using data across the entire frequency band to avoid omitting any signals. In addition, the calcu-