Laser enhanced high-intensity focused ultrasound thrombolysis: An *in vitro* study

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Abstract: Laser-enhanced thrombolysis by high intensity focused ultrasound (HIFU) treatment was studied *in vitro* with bovine blood clots. To achieve laser-enhanced thrombolysis, laser light was employed to illuminate the sample concurrently with HIFU radiation, and ultrasound and laser parameters were optimized to achieve better thrombolysis efficiency. The results indicated that the thrombolysis efficiency increased when pulse length of HIFU wave, HIFU pressure, or laser fluence increases. Also, with the presence of laser, an enhanced effect of thrombolysis was observed.

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1. Introduction

Catheter-based techniques have been extensively studied as an effective method to treat deep venous thrombosis (DVT) clinically.[1–6] However, these methods require a catheter to be placed within the blood clot, which is an invasive procedure. High intensity focused ultrasound (HIFU) is one of the non-invasive methods to treat abnormal tissues.[7–9] In pulsed-HIFU with low duty cycle, which is used for thrombolysis, energy deposition rates are low enough that temperature rises are well below the threshold for thermal damage. Thus the effects of clot disruption are obtained more mechanically, and cavitation effects are considered to make the biggest contribution during the process.[3,5,10–12] As a result, high acoustic peak negative pressure (as high as 19 MPa[13]) at relatively low ultrasound frequencies such as 500 kHz or 1 MHz are used so that cavitation can be effectively induced. However, at such low ultrasound frequencies, the focal spot of the ultrasound field can be larger than 10 mm in length, which is larger than the diameters of most veins. As a result, severe damage can occur to the surrounding tissue and vessel walls.

Both microbubbles and thrombolytic drugs have been applied during ultrasound-based thrombolysis. The addition of intravenously injected microbubbles can enhance the effects of ultrasound and reduce the required energy for producing ultrasound-mediated thrombolysis by decreasing cavitation threshold.[3,12,14,15] Also, a few of studies[16] have shown that HIFU can improve thrombolysis while applying thrombolytic agents by reducing the dosage of the drug and reducing the risk of hemorrhagic events, but increasing its efficacy. In addition, microbubbles and thrombolytic drugs can be combined together for targeted drug delivery, drug transport acceleration[17–21] and eventually enhancing thrombolysis. However, the use of microbubbles and thrombolytic drugs requires the systematic injection of foreign particles into the blood stream, and would have a lot concerns regarding the toxicity, efficiency, etc.[22]

In our recent study,[23] we found that when the laser system of photoacoustic imaging (PAI) was running simultaneously with HIFU treatment, an enhanced heating effect can be observed due to enhanced cavitation effects at a relatively low HIFU

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intensity. This finding potentially allows us to enhance HIFU without introducing foreign particles into the targeted tissue region. We expect that this discovery will be able to get a wider range of applications, such as thrombolysis, due to the enhanced cavitation effects.

In this study, we investigated the feasibility of laser-enhanced HIFU treatment in the *in vitro* thrombolysis application. The effects of acoustic and laser parameters (pulsed HIFU wave pulse length, pressure, and laser intensity) were assessed. More importantly, the thrombolysis efficiency was measured with and without laser illumination to explore the enhancement on HIFU thrombolysis.

2. Materials and methods

2.1 Experimental system

The overall experimental arrangement is shown in Fig. 1. The laser source with a pulse repetition rate of 10 Hz (5-ns pulse width) consists of a pulsed Q-switched, Nd:YAG laser and a tunable optical parameter oscillator (OPO) laser (Surelite OPO PLUS, Continuum, Santa Clara, CA). The generated laser beam was formed into a ring-shaped illumination on a condenser lens and refocused underneath a 5-MHz HIFU transducer (SU-108-013, Sonic Concepts, Bothell, WA), which was mounted on the condenser lens in the center hole. The 5-MHz HIFU transducer employed in this study has a 35 mm focal length and 33 mm active diameter with a $\sim$50% bandwidth. The focal point (-6 dB) of HIFU transducer is 2.61 mm $\times$ 0.51 mm $\times$ 0.51 mm in water. A 10-MHz focused ultrasonic transducer (V315, Olympus-NDT, Waltham, MA) (37.5 mm focal length; 70% -6-dB fractional bandwidth), which was placed at a 90-deg angle with the HIFU transducer and acted as a passive cavitation detector (PCD), was aligned to be confocal with the HIFU transducer and the laser beam prior to HIFU treatments. The HIFU source sine waves in burst mode were generated by a function generator (HP33250A, Agilent Technologies, Santa Clara, CA), and amplified by a 50-dB radio frequency amplifier (350L, ENI Technology, Inc., Rochester, NY), before they were sent to the HIFU transducer to induce cavitation in the sample. The PCD signal was received by a pre-amplifier (5072PR; Olympus-NDT, Waltham, MA) and captured by a data acquisition card (GageScope, CS21G8-256MSn Gage, Lockport, IL) in a personal computer. A10-MHz high-pass filter was used to remove contributions from the HIFU fundamental and second harmonic frequencies to ensure that the detected signals were mainly received from broadband acoustic emissions of cavitation. Both the 10- and 5-MHz transducers were immersed in a water tank filled with degassed water during the treatment.

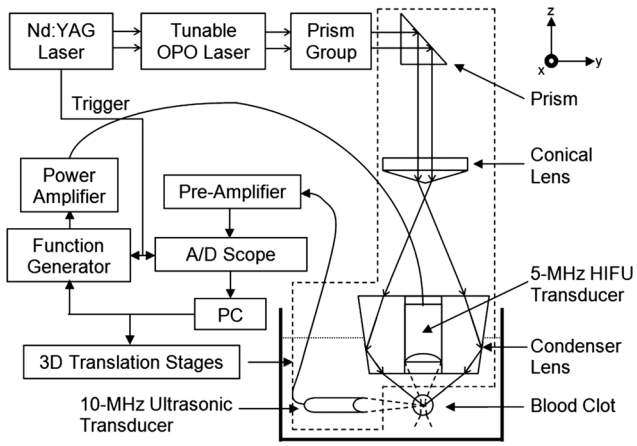

Fig. 1. System schematic.

2.2 Clot preparation

Whole bovine blood mixed with $CaCl_2$ in ~6 mm inner diameter tygon tubing was immersed in a 37 °C water bath for 2 h and then stored at 4 °C for up to 3 days to generate clots. Before the experiment, the clots cut into roughly cylindrical small pieces with a diameter of 5 mm and a length of 5 mm were placed in a tygon tubing (6.25 mm ID and 7.81 mm OD). The initial weight of blood clots were 205 ± 41 mg ($n = 155$).

2.3 Experiment procedure

All experiments were conducted in a water container filled with degassed water. Each blood clot were cut and weighted before the treatment and then placed in a clear tygon tubing for exposure under HIFU transducer and laser light. During the sonication, HIFU transducers, PCD detector and laser beams were driven by three-dimensional (3-D) translation stages along x, y, and z directions. The predetermined speed of the movement along x direction was 0.25 mm/s and the step size for y and z directions were set to 0.5 and 1 mm according to the size of the clot and HIFU transducer focal point. After the treatment, the clots were flushed with saline solution on a 400 μm pore size filter for weighting. The difference between the initial clot weight and the weight on the filter was used to calculate the weight loss, which is thrombolysis efficiency, and expressed by percentage.

2.4 Ultrasound and laser parameters

The experiments were performed in three groups. Each group contained only one changing variable (HIFU pulse length, HIFU pressure, or laser fluence) with the other parameters remained constant to examine the influence of each variable on thrombolysis efficiency. The treatment was repeated 5 times at each variable and performed both with and without laser illumination for comparison.

To obtain HIFU pressure at the focal point, HIFU transducer was calibrated in water using a standard hydrophone, and then a finite difference time domain (FDTD)[24] method was used to calculate the HIFU focal pressure. After these two steps, the relation between function generator voltage input and HIFU focal pressure can be found. In addition, the laser fluence at the sample surface was measured by a power meter prior to the experiment.

The duty cycle of HIFU waves for all treatments was 10%. The laser wavelength used in this study was 760 nm. During the treatment, the laser pulse with 10 Hz repetition rate and the generation of HIFU waves from the function generator were synchronized and triggered by the laser system. The pulsed laser and light wavelength in the near infrared region were chosen to maximize the penetration depth while the used laser intensity still complies with American National Standards Institute (ANSI) safety standard for laser use.

3. Results and discussion

The mean thrombolysis efficiency was plotted as a function of pulse length in Fig. 2(a). The HIFU negative pressure and laser fluence used in this group was 13.2 MPa and 27 mJ/cm^2, respectively. This laser fluence at 760 nm laser wavelength was slightly higher than the safety limit recommended by American National Standards Institute (ANSI),[25] which is 26.4mJ/cm^2. As we can see in the figure, longer HIFU pulse length yielded higher thrombolysis efficiency until it reached a saturation stage. With laser, the saturation point (800 μs pulse length) was reached earlier than it in the treatment without laser (1000 μs). In the rising stage of the curves, the thrombolysis efficiency was greatly enhanced by laser. At the pulse length of 800 μs, thrombolysis efficiencies with and without laser were 62.8% and 41.0%, and thus the enhancement by laser was about 53.1%. To further investigate the possible reason of this enhancement, acoustic cavitation signals were detected at each pulse length by the transducer. Figure 3 shows an example of PCD output as a function of time both with and without laser at 600 μs

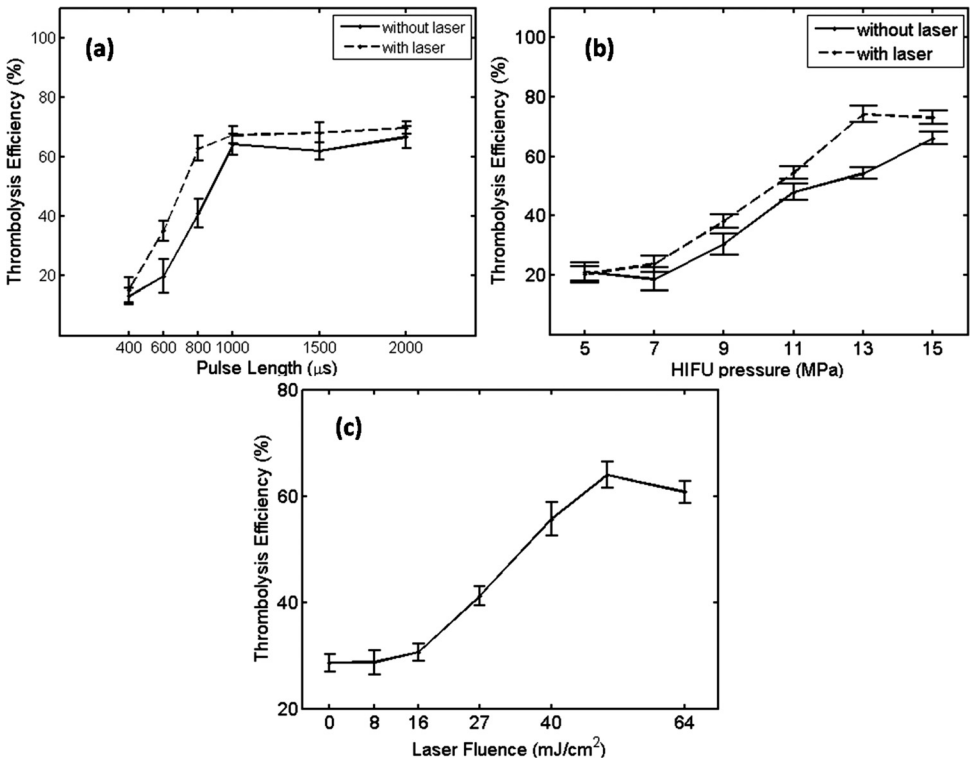

Fig. 2. Thrombolysis efficiency correlated with HIFU wave pulse length (a), HIFU pressures (b), and laser fluence (c) in the treatment with and without laser radiation.

HIFU pulse length. It is shown that cavitation signals are close to zero, and therefore no inertial cavitation occurred when laser was off. However, when laser was presented, the cavitation signals became much stronger, which indicated the occurrence of acoustic cavitation. After detecting PCD signals at each HIFU pulse length, it was observed that the cavitation occurred in the treatments with laser at 400 μs pulse length. However, without the laser illumination, the cavitation signals were very weak until the pulse length reached 1000 μs. As we know, cavitation has been demonstrated to enhance

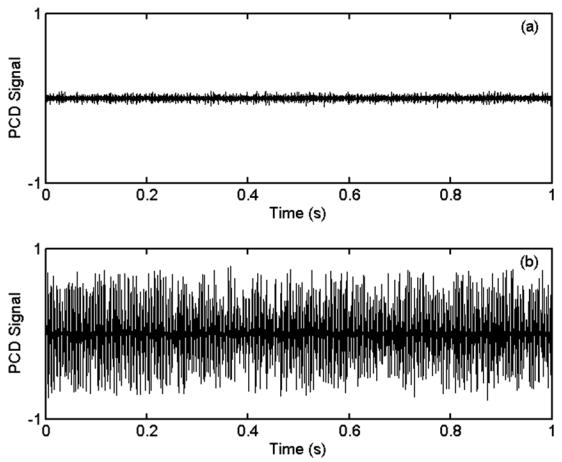

Fig. 3. PCD output as a function of time without (a) and with (b) laser at 600 μs HIFU wave pulse length.

thrombolysis effect. Therefore the possible reason of the enhancement of HIFU thrombolysis treatment in this study was the laser-enhanced acoustic cavitation.

To test the influence of HIFU negative pressure on thrombolysis efficiency, HIFU pulse length (1000 μs) and laser fluence(27 mJ/cm^2) were fixed. It is indicated in Fig. 2(b) that at 5 and 7 MPa HIFU negative pressure, the thrombolysis efficiency were only about 21.1% ± 3.0% and 18.6% ± 3.9%. However, as the HIFU pressure increases, a maximum of 74.2% ± 2.8% clot weight loss can be reached. Also, treatment with ultrasound alone produced less thrombolysis than that with the presence of laser. In addition, similar as the PCD detection in different HIFU pulse lengths measurements, acoustic cavitation can be observed at 7 and 9 MPa with and without laser. Thus, similarly, it is demonstrated again that the laser illumination has successfully lowered cavitation threshold and therefore enhanced the HIFU thrombolysis efficiency.

The effect of increasing laser fluence on thrombolysis was also examined as shown in Fig. 2(c). The HIFU negative pressure was 9.18 MPa and HIFU wave pulse length was 1000 μs in these treatments. Acoustic cavitation threshold was observed at laser fluence of 16 mJ/cm^2. When laser fluence was smaller than 16 mJ/cm^2, thrombolysis efficiency was low. However, as the laser fluence increased, a steep increase in thrombolysis efficiency was observed.

4. Discussion and conclusions

This study investigated the feasibility of using laser enhanced cavitation to enhance HIFU thrombolysis without the addition of any thrombolytic agents or microbubbles. Three important ultrasound and laser parameters (HIFU wave pulse length, HIFU pressure, and laser fluence) were chosen to test their influence on thrombolysis efficiency. As the pulse length, HIFU pressure, and laser fluence increases, better thrombolysis efficiency can be obtained. The optimal parameters were a pulse length of 1000 μs, HIFU negative pressure of 13 MPa and under 27 mJ/cm^2 laser radiation, at which a maximum thrombolysis efficiency (74.2% ± 2.8%) were achieved.

In addition, significant enhancement of thrombolysis efficiency was observed with laser illumination. Laser enhanced cavitation was considered to be a primary factor in the enhancement of HIFU thrombolysis treatment because it is shown that cavitation threshold was greatly reduced when laser was running concurrently with HIFU treatment. Generally, laser light may induce cavitation through heating or chemical breakdown. However, in our study, the laser fluence was under the ANSI safety limit and suggested that significant heating or chemical breakdown by laser light alone was unlikely. Therefore the combination of ultrasound and laser light could be the key factor for cavitation. A possible mechanism is that there is an instantaneous heating on the nano-second scale during laser illumination. When this instantaneous heating is coupled with negative ultrasound pressure, cavitation will be induced.

We have shown in our previous study[23] that laser enhanced HIFU treatment can be used in soft tissue thermal ablation. In this study, the results implied that this technique has successfully shown another potential application in the thrombolysis field. More future work is needed to evaluate its *in vivo* and clinical feasibility and potential risk of injury of vessel wall and surrounding tissue.

Acknowledgments

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