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Sonothromblysis in Combination with Thrombolytic Drugs in a Rabbit Model Using MRI-Guidance

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ABSTRACT

The potential of MRI-guided focused ultrasound (MRgFUS) combined with the thrombolytic drug recombinant tissue plasminogen activator (rt-PA), to dissolve clots in the carotid of a New Zealand rabbit *in vivo* is evaluated. A spherically-focused transducers of 5 cm diameter; focusing at 10 cm and operating at 1 MHz was used. A pulsed ultrasound protocol was used that maintains a tissue temperature increase of less than 1˚C in the clot (called safe temperature). MRgFUS has the potentials to dissolve clots that are injected in the carotid of rabbits *in vivo*. It was found that the time needed for opening the carotid artery using ultrasound and rt-PA was decreased compared to just using rt-PA. The proposed protocol was monitored using Magnetic Resonance Angiography (MRA) every 1 min.

Keywords: Ultrasound; MRI; Stroke; Thrombolysis

1. Introduction

The feasibility of ultrasound to enhance thrombolysis was reported in the mid 70s [1,2]. In the following years, several *in vitro* studies (Kimura *et al*. 1994 [3] and Spengos K *et al*. 2000 [4]) have confirmed the above results. In the former studies, the range of intensity varied from 0.2 - 2.0 W/cm^2 (spatial peak temporal average intensity) and the frequency from 20 kHz to 2 MHz using unfocused ultrasound.

It is speculated that ultrasound accelerates enzymatic fibrinolysis primarily through mechanical mechanisms, by enhancing the effectiveness of thrombolytic drugs, possibly by exposing more binding sites on the fibrin to the recombinant tissue plasminogen activator (rt-PA) [5]. Other mechanical effects of ultrasound, such as cavitation and radiation force, are possibly influencing drug transport [6,7]. Acoustic cavitation plays a very significant role in ultrasound-accelerated fibrinolysis [6]. Other theories revolve around the fact that ultrasound promotes motion of fluid around the clot, an effect called microstreaming [7].

Birnbaum *et al*. in 1998 [8], reported that *in vivo* arterial clot dissolution can be achieved with intravenous microbubbles and transcutaneous ultrasound delivery alone. Moreover, a study has shown the effectiveness of transcutaneous ultrasound and intravascular microbubbles in lysing intracranial clot in pigs [9].

Administration of gaseous microspheres dramatically lowers the threshold for cavitation and increases the lytic activity of ultrasound (Holland CK and Aplef RE 1990[10]). Because the bubbles are destroyed in the process, they must be constantly injected for complete clot dissolution. Recent studies have demonstrated that microbubbles can be concentrated at the surface of clot by attaching a glycoprotein receptor antagonist, which increases their adherence to acute clot, to the bubbles [11].

The use of Focused ultrasound for destroying clots has received attention lately (Frenkel V *et al*. 2006 [12]; Shawa G *et al*. 2009 [13]; Hölscher T *et al*. 2011[14] and Laing S *et al*. 2011[15]). In the study by Frenkel V *et al*. 2006 [12], clots from humans *in vitro* were ablated using pulse ultrasound with a 1 MHz single element transducer in synergy with rt-PA. In this study the conclusion is that by using rt-PA and focused ultrasound, improved clot destruction rates were achieved. In another study, using unfocused ultrasound at 120 KHz [12], showed that ultrasound has the potential of destroying clots *in vitro* in combination with Liposomes loaded with rt-PA. Human whole blood clots were ablated though the skull using a hemispheric phased array transducer with 1,000 single piezo elements [13] using pulse ultrasound. The conclusion of this study is that ultrasound alone can be utilized to destroy clots.

In addition to these *in vitro* studies, there are some in-

teresting *in vivo* studies confirming the therapeutic effect of pulse ultrasound (Hölscher T. *et al*. 2011 [14] and Laing S *et al*. 2011 [15]). In one study (Laing S *et al*. 2011 [15]), a rabbit aorta model was used in order to ablate using pulse ultrasound in combination with Liposomes loaded with rt-PA. In another study (Hölscher T. *et al*. 2011 [14]), clots that were formed in the rabbit marginal ear vein were ablated using pulse ultrasound with a 1 MHz single element transducer in synergy with recombinant tissue plasminogen activator (rt-PA).

Currently there are few but significant clinical trials: a) The CLOTBUST (Combined Lysis of Thrombus in Brain ischemia using transcranial Ultrasound and Systemic Recombinant Tissue-Type Plasminogen Activator (rt-PA)) is a Phase II randomized multi-center international clinical trial [16]; b) The EKOS clinical trial [17] which involves the insertion of a catheter is now being tested in phase II-III Interventional Management of Stroke (IMS) trials; c) The TRUMBI clinical trial (Daffertshofer M *et al*. 2005 [18]), using transcranial low-frequency ultrasound-mediated thrombolysis in brain ischemia in combination with intravenous administration of tPA; and d) The TUCSON clinical trial (Barreto A *et al*. 2009 [19]), a phase I-II randomized placebo-controlled, international multi-center study, using perfultren-lipid microspheres.

In this paper, the therapeutic effect of focused ultrasound and administration of rt-PA to dissolve artificial clot that was injected in the carotid artery of a New Zealand rabbit was examined. In the current study, higher intensities were used than what was proposed in a study by Alexandrov AV *et al*. in 2004 [20]. We anticipate that by using higher intensities, the rate of clot destruction will be accelerated. The previous studies mostly make use of unfocused ultrasound (Holland CK *et al*. 2008 [21]; Saguchi T *et al*. 2008 [22] and Jürgen E *et al*. 2008 [23]). Thus, the intensity levels that could be used were limited. In this study, focused ultrasound is investigated and therefore higher intensities are utilized provided that thermal effects are avoided. Focused ultrasound targeting the clot is used with spatial average temporal average (SATA) *in situ* intensity of 20 W/cm^2.

2. Materials and Methods

2.1. HIFU/MRI System

Figure 1 shows the block diagram of the MRgFUS which includes the following subsystems: 1) Focused ultrasound system, 2) MRI system, 3) Transducer holder and 4) Temperature measurement. Since this system eventually will be utilized in conjunction with MRI the transducer and transducer holder are both designed to be MRI compatible. We have chosen to use MRI, because regarding vascular imaging is considered the gold standard.

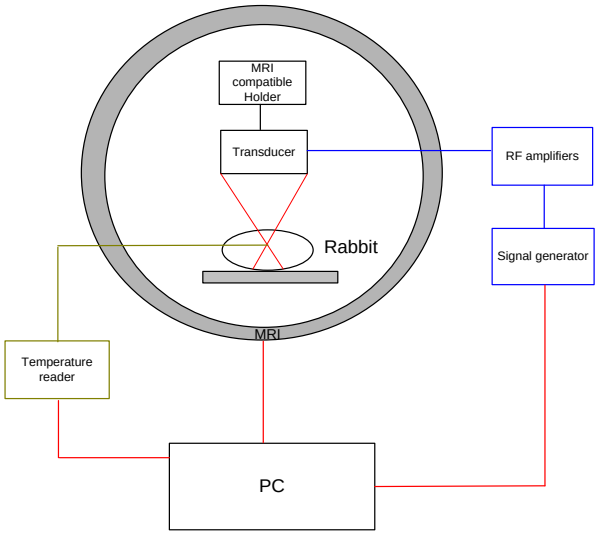

Figure 1. MRgFUS system for *in vitro* sonothrombolysis.

2.2. Focused Ultrasound System—MR Imaging

The ultrasound system consists of a radio frequency (RF) generator/amplifier (1000 W, JJ&A Instruments, Duvall, WA, USA), and a spherically shaped transducer made from piezoelectric ceramic of low magnetic susceptibility (Piezotechnologies, Etalon, Lebanon, IN, USA). The transducer used operates at 1 MHz. The transducer has a focal length of 10 cm and a diameter of 5 cm. The transducer is coupled to the artery using a special designed plastic holder (MEDSONIC, Limassol, Cyprus). The transducer and transducer holder were placed inside an MRI scanner (Signa 1.5 T, by General Electric, Fairfield, CT, USA).

2.3. Production of Sample Clots

Blood clots were obtained by natural coagulation of animal blood samples from healthy cows. The animal experiments protocol was approved by the national body in Cyprus responsible for animal studies (Ministry of Agriculture, Animal Services). Blood was drawn into small containers and placed in a 37˚C water bath for 3 h and then stored in a refrigerator at a temperature of 5˚C for at least 72 h before use in the experiments to allow complete clot retraction (Holland CK *et al*. 2008 [21]).

2.4. Preparation of rt-PA

The rt-PA was obtained as a lyophilized powder (rt-PA, Actilase, Genentech, San Francisco, CA, USA) mixed with sterile water as per manufacturer's instructions. A dose of 1 mg/mL was administered.

2.5. MRI Parameters

For T2-W FSE the following parameters were used: TR

= 2500 ms, echo time (TE) was variable from 10 ms to 160 ms, slice thickness = 3 mm (gap 0.3 mm), matrix = 256 × 256, FOV = 16 cm, NEX = 1, and ETL = 8. For the MRI sequence of MRA the following parameters were used: repetition time (TR) = 40 ms, echo time (TE) = 2.7 ms, Field of View (FOV) = 16 cm, matrix = 256 × 256, flip angle = 10˚, Bandwidth = 15.6 KHz, Number of excitations (NEX) = 1. A spinal coil (USA instruments, Cleveland, OH, USA) was used to acquire the MRI signal.

2.6. *In Vivo* Experiments

For the *in vivo* experiments, 16 New Zealand adult rabbits were used weighting approximately 3.5 - 4 kg. The rabbits were anaesthetized using a mixture of 500 mg of ketamine (100 mg/mL, Aveco, Ford Dodge, IA), 160 mg of xylazine (20 mg/mL, Loyd Laboratories, Shenandoah, IA), and 20 mg of acepromazine (10 mg/mL, Aveco, Ford Dodge, IA) at a dose of 1 mL/kg. The animal experiments protocol was approved by the national body in Cyprus responsible for animal studies (Ministry of Agriculture, Animal Services).

For the *in vivo* experiments, the clot was injected in the carotid artery using a thin needle. Once the blockage of the artery was confirmed, ultrasound and rt-PA therapy was applied. A1 mg/ml/kg rt-PA was injected in the jugular vein. Prior to the application of ultrasound a bolus of an ultrasound contrast agent (SonoVue; Bracco SpA, Milan, Italy) was injected intravenously through the ear vein at a dose of 0.02 mL/kg.

3. Results

Figure 2(a) shows the coupling of the transducer to the carotid artery and **Figure 2(b)** shows the MRI image using T2-W of the coupling of the ultrasonic transducer to the carotid artery of the rabbit. The intense signal indicates the water of the transducer holder which makes an excellent coupling to the rabbit.

Figure 4(a) shows the carotid artery using MRA before the injection of the thrombus. **Figure 4(b)** shows the MRA image of the carotid artery immediately after the injection of the thrombus. **Figure 4(c)** shows the MRA image of the carotid artery after applying Ultrasound (f = 1 MHz, SATA intensity = 20 W/cm^2, Duty factor = 10%, pulse repetition rate = 10 Hz,) and rt-PA for 70 mins (the artery is completely opened).

4. Discussion

The results in this study demonstrate the ability of MRgFUS in combination with rt-PA to dissolve clot**s** in an *in vivo* model. We have proved the capability of the clot model (thrombus) to block the carotid artery and also

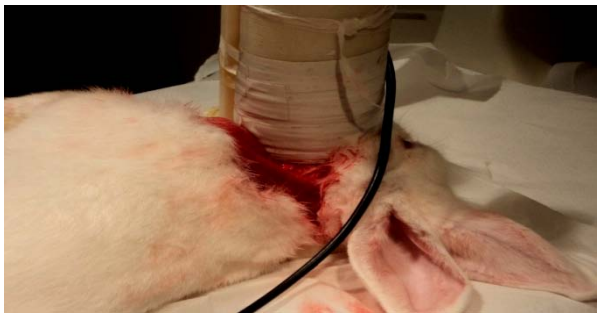

Figure 2. (a). Transducer coupling to the artery (b) MR image using T2-W FSE for the coupling of the ultrasonic transducer to the neck of the rabbit.

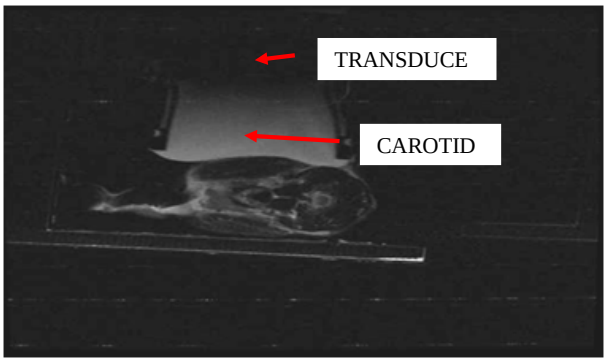

Figure 3. Shows the coupling of the transducer to the carotid artery.

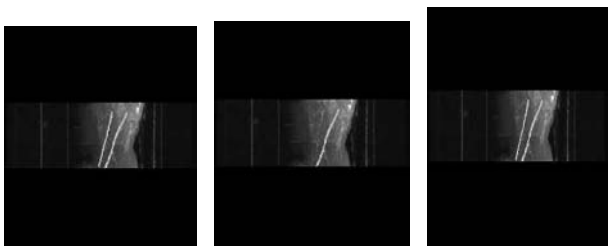

Figure 4. (a) MRA image of carotid artery before the injection of the thrombus; (b) MRA image of carotid artery immediately after the injection of the thrombus; (c) MRA image of the carotid artery after applying Ultrasound and rt-PA for 70 min.

proved the ability of therapeutic ultrasound in synergy with rt-PA to dissolve clots and at the same time monitoring the whole process using MRA. We have chosen to use the carotid artery because most of the arteries in such a small animal such us the rabbit cannot be visualized with MRI due to spatial resolution problems (for example ear, femoral, middle cerebral artery etc).

The results of this study clearly show that focused ultrasound has the potential to accelerate the treatment of vascular occlusions (potential application could be ischemic stroke) by reducing the treatment time which is crucial for future clinical treatments (ischemic stroke for

example). By now, the administration of rt-PA is an effective treatment for acute ischemic stroke, but it is associated with an increased risk of intracranial haemorrhage. Therefore, a potential benefit from the application of focused ultrasound is to use lower rt-PA dose without affecting the functionality of the treatment, and ensuring no haemorrhage is caused due to rt-PA.

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