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● *Original Contribution*

EVALUATION OF DOSE DISTRIBUTION OF MOLECULAR DELIVERY AFTER BLOOD-BRAIN BARRIER DISRUPTION BY FOCUSED ULTRASOUND WITH TREATMENT PLANNING

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Abstract—The permeability of the blood-brain barrier (BBB) can be enhanced by focused ultrasound (FUS) in localized regions with applications of ultrasound contrast agent (UCA). The purpose of this study was to evaluate the dose distribution of Evans blue (EB) in the targeted brain by sonication with treatment strategy. FUS exposure was applied with an ultrasound frequency of 1 MHz, a 5% duty cycle and a repetition frequency of 1 Hz. Single sonication with two doses of UCA and two sonications at the same location or an interval of 3 mm to induce BBB disruption for assessing dose distribution. The permeability of the BBB was measured quantitatively based on EB extravasation. Gadolinium deposition was monitored by contrast enhanced MR imaging for dose distribution of the focal plane. Hematoxylin and eosin staining was performed for histologic observation. No significant difference was found for EB in the focal regions between the single sonication with UCA at a dose of 300 μL/kg and repeated sonication with UCA at a lower dose of 150 μL/kg. There was a sharper dose distribution in the brain with repeated sonication at the same location, compared with the brain receiving two sonications at an interval of 3 mm. Compared with a single sonication with UCA at a dose of 150 μL/kg, the histologic evaluation of the sonicated regions indicated that more erythrocytes were seen in the brain treated with single sonication at a higher dose of 300 μL/kg or repeated sonication at a dose of 150 μL/kg. This study demonstrated that the dose distribution of molecular delivery could be regulated by sonication with treatment planning. (E-mail: fyyang@ym.edu.tw) © **2013 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Focused ultrasound, Multiple sonications, Blood-brain barrier disruption, Dose distribution, Treatment planning.**

INTRODUCTION

The blood-brain barrier (BBB) keeps the brain healthy, and its impermeability makes the treatment of brain diseases using chemotherapy difficult. The BBB is a key factor involved in the delivery of drugs to the human brain (Pardridge 2005). Chemical modification of potent agents is able to increase permeability, but the drugs may have undesired dose-limiting side effects due to their spread over a large volume within the brain. The combination of focused ultrasound (FUS) and ultrasound contrast agent (UCA) may provide a non-invasive, transient and localized method for opening the BBB (Hynynen et al. 2001; Hynynen et al. 2005).

Passive drug release into tumor tissues following extravasation from leaky tumor vessels allows most drug delivery systems to target tumor tissue (Allen and Cullis 2004). Liposome encapsulated drugs are used to increase drug levels in tumors, in comparison with drugs used to treat normal tissues. Some chemical formulations use specific ligands or antibodies to enhance intracellular uptake of these carriers (Park et al. 2002). Different protocols for hyperthermia-mediated drug release from liposomes could permit intra-tumoral drug distribution to be controlled in real time. Drug accumulation patterns may influence anti-tumor efficacy (Ponce et al. 2007); hence, uniform temperature regulation in this technology

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is important in treating tumors of different sizes and shapes.

Physiologic barriers interfere with therapeutic drug concentration and keep drugs from entering tumors (Floyd et al. 2005). FUS and UCA significantly enhance delivery of liposomal doxorubicin into brain tumors and improve anti-tumor effects, as shown in previous studies (Yang et al. 2012a, b, c, d; Yang et al. 2012a, b, c, d). The aforementioned repeated sonications could further increase the efficiency of delivery (Yang et al. 2011a, b, c). One study reports spatial and temporal variations of FUS-induced trans-BBB molecular delivery (Choi et al. 2007). Relationships between sonication strategies and drug delivery patterns are thought not to have been investigated, as of the writing of this paper. The efficacy of chemotherapeutics will be increased while this technology is able to reach the efficient delivery of a therapeutic level to a planned target volume. A larger target volume could be sonicated at multiple locations for BBB disruption. Additionally, sharp focal dose distribution of drugs may be introduced deep in the brain with repeated sonications at the same region.

On the basis of these promising results, there may be great value in defining a treatment strategy for chemotherapy in brain tumors with FUS. The objective of this study is to investigate the dose distribution of molecular delivery in the brain with BBB disruption induced by FUS exposure at various treatment protocols.

MATERIALS AND METHODS

Preparation of animals

All procedures were approved according to guidelines stipulated by the Animal Care and Use Committee of the National Yang Ming University. Male Sprague-Dawley rats weighing from 280 to 350 g were used in this study. Before FUS exposure, the animals were anesthetized intra-peritoneally with chloral hydrate (400 mg/kg), and the body temperature was maintained at 37°C using a heating pad. The rat heads were mounted on a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The top of the cranium was shaved, and the scalp overlying the skull was incised to identify the bregma as an anatomic landmark for targeting. Ultrasound transmission gel (Pharmaceutical Innovations, Newark, NJ, USA) was used to cover the area between the transducer and the skull to maximize ultrasound transmission.

Pulsed ultrasound equipment

The schematic diagram of the pulsed FUS system for sonication is shown in Figure 1. Ultrasound exposure was generated by a FUS transducer (H101MR, Sonic Concepts, Bothell, WA, USA) with a 64 mm diameter, a 62.64 mm radius of curvature and a resonant frequency of 1 MHz. The half maximum pressure amplitude diameter and length of the focal spot were 1.5 and 8 mm, respectively. The transducer was mounted on a removable cone filled with degassed water that had its tip sealed with a polyurethane membrane. The procedure is the same as mentioned in previous works undertaken by the authors of this paper (Yang et al. 2009; Yang et al. 2010). UCA (SonoVue, Bracco International, Amsterdam, The Netherlands) was injected into the femoral vein of the rats about 15 s before each sonication. The UCA used in this procedure contained phospholipid-coated microbubbles with a mean diameter of 2.5 μm, at a concentration of 1 x 10^8 to 5 x 10^8 bubbles/ml. Pulsed FUS was applied with a burst length of 50 ms at a 5% duty cycle

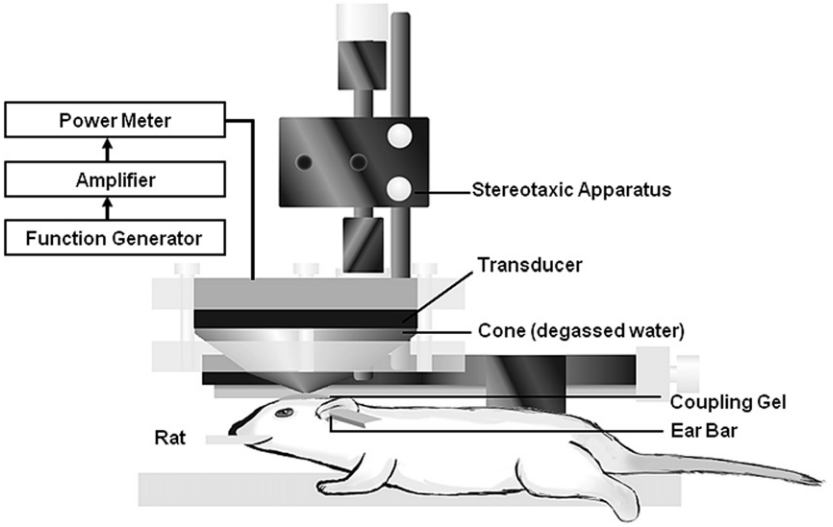

Fig. 1. Schematic diagram of a focused ultrasound setup for blood-brain barrier disruption. The transducer was attached to a stereotaxic apparatus.