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● *Original Contribution*

DELIVERY OF LIPOSOMES WITH DIFFERENT SIZES TO MICE BRAIN AFTER SONICATION BY FOCUSED ULTRASOUND IN THE PRESENCE OF MICROBUBBLES

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Abstract—Imaging or therapeutic agents larger than the blood–brain barrier's (BBB) exclusion threshold of 400 Da could be delivered locally, non-invasively and reversibly by focused ultrasound (FUS) with circulating microbubbles. The size of agents is an important factor to the delivery outcome using this method. Liposomes are important drug carriers with controllable sizes in a range of nanometers. However, discrepancies among deliveries of intact liposomes with different sizes, especially those larger than 50 nm, across the BBB opened by FUS with microbubbles remain unexplored. In the present study, rhodamine-labeled long-circulating pegylated liposomes with diameters of 55 nm, 120 nm and 200 nm were delivered to mice brains after BBB disruption by pulsed FUS with microbubbles. Four groups of peak rarefactional pressure and microbubble dosages were used: 0.53 MPa with 0.1 μL/g (group 1), 0.53 MPa with 0.5 μL/g (group 2), 0.64 MPa with 0.1 μL/g (group 3) and 0.64 MPa with 0.5 μL/g (group 4). The delivery outcome was observed using fluorescence imaging of brain sections. It was found that the delivery of 55-nm liposomes showed higher success rates than 120-nm or 200-nm liposomes from groups 1–3. The result indicated that it may be more difficult to deliver larger liposomes (>120 nm) passively than 55-nm liposomes after BBB opening by FUS with microbubbles. The relative fluorescence area of 55-nm liposomes to the total area of the sonicated region was statistically larger than that of the 120-nm or 200-nm liposomes. Increasing peak rarefactional pressure amplitude or microbubble dose could induce more accumulation of liposomes in the brain using FUS with microbubbles. Moreover, the distribution pattern of delivered liposomes was heterogeneous and characterized by separated fluorescence spots with cloud-like periphery surrounding a bright center, indicating confined diffusion in the extracellular matrix after extravasation from the microvasculature. These findings are expected to provide useful information for developing FUS with microbubbles as an effective trans-BBB liposomal drug delivery strategy. (E-mail: danguo@szu.edu.cn) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Focused ultrasound, Microbubbles, Blood–brain barrier, Delivery, Liposomes, Different size.**

INTRODUCTION

It has been a great challenge to deliver therapeutic drugs to the brain due to the existence of the blood–brain barrier (BBB) (Pardridge 2005). Animal studies have demonstrated that the BBB endothelial lining or tight junctions could be opened locally, non-invasively and reversibly by focused ultrasound (FUS) in the presence of microbubbles in recent years (Choi et al. 2007; Hynynen et al. 2001; Shen et al. 2014). This technique has provided potential availability in targeted drug delivery for brain diseases (Aryal et al. 2014).

There have been many animal studies conducted on the delivery of various imaging molecules or therapeutic agents across the BBB using FUS or unfocused ultrasound with circulating microbubbles, such as Evans bluedye (Beccaria et al. 2013), magnetic resonance

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imaging (MRI) contrast agents (Hynynen et al. 2001), dextrans (Chen and Konofagou 2014; Choi et al. 2010b), chemotherapy agents (Aryal et al. 2013; Treat et al. 2007; Treat et al. 2012; Yang et al. 2012), antibodies (Kinoshita et al. 2006), magnetic nanoparticles (Fan et al. 2013), gene therapy vectors (Alonso et al. 2013), stem cells (Burgess et al. 2011) *etc*. A recent study conducted on rhesus macaques demonstrated that BBB disruption could be achieved with a probability of 50% using 220 kHz FUS with 10-ms bursts of 1 Hz repetition frequency at 0.15 MPa for 70 s without evident histologic or functional damage (McDannold et al. 2012). Another study conducted on two male rhesus monkeys showed that targeted and safe BBB opening could be achieved by a single-element 500-kHz spherical transducer ultrasound system with a real-time monitoring technique based on cavitation spectral analysis (Marquet et al. 2014). These results supported the feasibility of this unique non-invasive and targeted drug delivery approach of the central nervous system.

Extensive attention was paid to investigate delivering various drugs to treat animals with brain diseases using ultrasound with microbubbles, and several studies found that the molecular weight (MW) of an agent had obvious influence on delivery to brain. In the experiment on rhesus macaques, lower-level signal enhancement was observed with gadofosveset trisodium (MW: 67 kDa), an MRI contrast agent that binds to albumin in the blood, when compared to Gd-DPTA, another MRI agent with a much smaller molecular weight (MW: 938 Da) (McDannold et al. 2012). A fluorescence imaging study by Chen and Konofagou (2014) showed that fluorescent dextrans with MWs up to 2000 kDa could be delivered to mice brains, but needed a much higher pressure amplitude (0.84 MPa) than that for delivery of 3-kDa dextrans. These results suggested that the MW of particles is an important factor to their extravasation from brain microvasculature into parenchyma using ultrasound with microbubbles. However, the effect of the size of agents, an important characteristic of some drug carrier systems like liposomes, on the delivery outcome using ultrasound with microbubbles has not been widely investigated.

Liposomes (enclosed phospholipid bilayer structures) are proposed as drug carrier systems to deliver many drugs, including anti-cancer, anti-fungal and antibiotic drugs (Allen and Cullis 2013). They have been used to improve the therapeutic efficiency of drugs by enhancing drug accumulation, prolonging biological half-life or reducing toxicity. For example, Doxil is an anti-cancer liposomal drug with a diameter of 80–100 nm that showed enhanced therapeutic effect on extracranial cancers, such as breast and ovarian cancers, over free doxorubicin (Barenholz 2012; Gabizon et al. 1994; Symon et al. 1999). However, liposomal drugs are impeded by the BBB because they are usually too large to cross (Allen and Cullis 2013; Pardridge 2005). Although many studies demonstrated that large molecules could be delivered to the brain by ultrasound with microbubbles, the delivery of intact liposomes, especially those larger than 50 nm, across the BBB opened by ultrasound combined with microbubbles remains uninvestigated. Specifically, the discrepancies among liposomes with different sizes moving across the BBB using this method are unknown.

The main purpose of this study was to investigate delivery of intact liposomes with different sizes after the BBB opening was induced by FUS in the presence of circulating microbubbles. Effects of different pressure amplitudes and microbubble dosages on delivery outcomes were also studied. In particular, fluorescently labeled stabilized long-circulating pegylated (PEG) liposomes with three sets of diameters of 55 nm, 120 nm and 200 nm were prepared and administered intravenously after FUS sonication. Normal mice were sonicated with different peak rarefactional pressures and microbubble dosages. The evaluation of delivery outcome was performed using fluorescence imaging of brain slices. Histologic analysis was carried out to examine tissue damaging effects.

MATERIALS AND METHODS

Animal preparation

Experiments were performed on 76 female BALB/c mice (Guangdong Medical Laboratory Animal Center, Foshan, China) (6-wk-old; 20 ± 2 g). They were housed in sterile isolated cages with a 12 h light/dark cycle at constant temperatures (24–26°C) and humidity (30–50%). Animal care and experiments were approved by the Animal Care and Use Committee of School of Medicine in Shenzhen University.

Before sonication, the mice were anesthetized with 1.5% isoflurane (RWD Life Science, Co.,Ltd., Shenzhen, China), and placed on a heating pad with a constant temperature of 37°C to maintain their body temperature. Then, the hair on the scalp of each mouse was removed with depilatory cream.

In our preliminary experiment, the BBB could be opened successfully and non-invasively without any hemorrhage and neuron damaging effects at a peak rarefactional pressure of 0.53 MPa and with a microbubble dosage of 0.1 μL/g. In order to explore the effects of the parameters on the delivery outcomes of liposomes, four groups of peak rarefactional pressures and microbubble dosages were used in the present study: 0.53 MPa with 0.1 μL/g (group 1), 0.53 MPa with 0.5 μL/g (group 2), 0.64 MPa with 0.1 μL/g (group 3) and 0.64 MPa with 0.5 μL/g (group 4). The experiment groups are shown in Table 1. For each group, 18 mice were sonicated transcranially using FUS with