Ultrasound in Med. & Biol., Vol. 40, No. 1, pp. 130–137, 2014

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2013.09.015

● *Original Contribution*

MICROBUBBLE TYPE AND DISTRIBUTION DEPENDENCE OF FOCUSED ULTRASOUND-INDUCED BLOOD–BRAIN BARRIER OPENING

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(*Received* 19 *November* 2012; *revised* 10 *September* 2013; *in final form* 11 *September* 2013)

Abstract—Focused ultrasound, in the presence of microbubbles, has been used non-invasively to induce reversible blood–brain barrier (BBB) opening in both rodents and non-human primates. This study was aimed at identifying the dependence of BBB opening properties on polydisperse microbubble (all clinically approved microbubbles are polydisperse) type and distribution by using a clinically approved ultrasound contrast agent (Definity microbubbles) and in-house prepared polydisperse (IHP) microbubbles in mice. A total of 18 C57 BL/6 mice (n = 3) were used in this study, and each mouse was injected with either Definity or IHP microbubbles *via* the tail vein. The concentration and size distribution of activated Definity and IHP microbubbles were measured, and the microbubbles were diluted to 6×10^8/mL before injection. Immediately after microbubble administration, mice were subjected to focused ultrasound with the following parameters: frequency = 1.5 MHz, pulse repetition frequency = 10 Hz, 1000 cycles, *in situ* peak rarefactional acoustic pressures = 0.3, 0.45 and 0.6 MPa for a sonication duration of 60 s. Contrast-enhanced magnetic resonance imaging was used to confirm BBB opening and allowed for image-based analysis. Permeability of the treated region and volume of BBB opening did not significantly differ between the two types of microbubbles ($p > 0.05$) at peak rarefractional acoustic pressures of 0.45 and 0.6 MPa, whereas IHP microbubbles had significantly higher permeability and opening volume ($p < 0.05$) at the relatively lower pressure of 0.3 MPa. The results from this study indicate that microbubble type and distribution could have significant effects on focused ultrasound-induced BBB opening at lower pressures, but less important effects at higher pressures, possibly because of the stable cavitation that governs the former. This difference may have become less significant at higher pressures, where inertial cavitation typically occurs. (E-mail: ek2191@columbia.edu) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Blood-brain barrier opening, Focused ultrasound, Microbubble type, Microbubble distribution.**

INTRODUCTION

One of the main obstacles in the treatment of neurodegenerative diseases (*e.g.,* Parkinson's disease, Alzheimer's disease) is the blood-brain barrier (BBB). Although the primary function of the BBB is to prevent toxins from entering the brain parenchyma, it also impedes the delivery of therapeutic agents ≥400 Da (Pardridge 2005). Different strategies have been proposed to temporarily disrupt the BBB, including hyper-osmolar solutions (such as mannitol) and focused ultrasound (FUS) in combination with microbubbles. In contrast to the hyper-osmolar methods, FUS in the presence of microbubbles was found to be the only non-invasive approach capable of temporarily opening the BBB in the targeted region (Choi et al. 2007; Hynynen et al. 2001). With the use of carefully selected acoustic parameters, FUS-induced BBB opening was found to be safe in both rodents (Baseri et al. 2010) and non-human primates (Marquet et al. 2011; McDannold et al. 2012; Tung et al. 2011a).

Although the exact mechanism is still not completely understood, the interaction between capillary walls and acoustically driven microbubbles was found to be one of the key factors that lead to disruption of the BBB (Tung et al. 2011b). Until now, most studies have used commercially available and U.S. Food and Drug Administration-approved ultrasound contrast agents (UCAs). These include the protein-coated UCA Optison (Choi et al. 2007; McDannold et al. 2008) and the lipid-coated UCA Definity (McDannold et al. 2012; Tung et al. 2011b). Compared with protein-coated

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microbubbles, lipid-based microbubbles are formed by self-assembled monolayer phospholipids and are more responsive to ultrasound (Sirsi and Borden 2009). Definity microbubbles are highly polydisperse agents with bubble diameters ranging from submicrons to >10 μm. As a result, the resonant frequencies of these bubbles cover a wide range on the spectrum (>10 MHz) (Cheung et al. 2008; Goertz et al. 2007). Using Definity microbubbles, Baseri et al. (2010) evaluated the BBB opening threshold and most tolerable acoustic pressure ranges in mice at 1.525 MHz. The acoustic pressure window of 0.3–0.46 MPa was determined to be tolerable at the parameters used in that study (pulse length = 20 ms, pulse repetition frequency = 10 Hz).

In the study described here, we aimed to compare Definity and in-house prepared polydisperse (IHP) microbubbles, both of which are formed by high shear gas dispersion in an aqueous lipid-shell mixture. Although these two microbubbles have similar compositions, their behavior in the application of FUS-induced BBB opening has not been studied. The two main goals of this study were: (i) to evaluate whether Definity and IHP microbubbles have similar effects on BBB opening properties, and (ii) to determine whether IHP can serve as a surrogate for the commercially available Definity microbubbles. The efficiency of BBB opening using these microbubbles was evaluated by analyzing the increase in brain tissue permeability and the total volume of BBB opening. Microbubble type dependence was evaluated at different *in situ* acoustic pressures, ranging from 0.3 to 0.6 MPa. Tung et al. (2010a) reported (using Definity microbubbles) that inertial cavitation occurred at 0.45 and 0.6 MPa, but not at 0.3 MPa, in mice. Therefore, the FUS parameters selected in this study covered both stable and inertial cavitation regimes for Definity microbubbles. Finally, BBB reversibility was monitored and histologic observations of the brains were performed for evaluation of tolerability.

METHODS

Microbubbles

As indicated previously, two types of microbubbles were used in this study: Definity (Lantheus Medical Imaging, North Billerica, MA, USA) and IHP microbubbles. Definity vials, which are composed primarily of an aqueous solution of lipids and octofluoropropane (C_3 F_8) gas, were stored at 4°C before use. Immediately before sonication, Definity vials were activated (at an initial temperature of 4°C) *via* mechanical agitation using a VialMix (Lantheus Medical Imaging) shaker for a pre-set time of 45 s. The IHP microbubbles were manufactured according to a previously published protocol (Feshitan et al. 2009). Briefly, 1, 2-distearoyl-*sn*-glycero-3-phosphocholine and polyethylene glycol 2000 were mixed at a 9:1 ratio. Ten milligrams of the mixture was dissolved in a 10-mL solution consisting of filtered phosphate-buffered saline/glycerol (10% volume)/propylene glycol (10% volume) using a sonicator (Model 1510, Branson Ultrasonics, Danbury, CT, USA). Each IHP microbubble vial (total volume = 5 mL) contained 2 mL of lipid solution, and the vial was sealed and stored at 4°C. Before activation, the air in the IHP vial was vacuumed out *via* a 26-gauge needle and the head space of the vial was filled with decafluorobutane (C_4 F_{10}) gas. This vacuuming-filling procedure was repeated five times for each vial to ensure high C_4 F_{10} concentration. The vial was then activated *via* a VialMix shaker for 45 s.

Immediately after activation, the concentration and size distribution of each microbubble vial were measured with a Coulter Counter Multisizer (Beckman Coulter, Fullerton, CA, USA), which measures microbubbles in the range 0.6–18 μm. The microbubbles were then diluted in sterile saline (Vedco, Saint Joseph, MO, USA), yielding a concentration of approximately 6×10^8 bubbles/mL.

Preparation of animals

A total of 18 mice (C57BL/6, Harlan, Indianapolis, IN, USA) were used in this study. Each mouse was anesthetized with a mixture of oxygen and 1%–2% isoflurane (SurgiVet, Smiths Medical PM, Dublin, OH, USA) and placed prone with its head immobilized by a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The hair on the mouse head was removed with an electric trimmer and depilatory cream to minimize impedance mismatch. All procedures involving animals were approved by the Columbia University Institutional Animal Care and Use Committee.

Sonication protocol and MRI

A single-element FUS transducer (focal length = 60 mm, radius = 30 mm, Imasonic, Voray/l'Ognon, France) with a center frequency of 1.5 MHz was used for all sonications. A pulse-echo transducer (radius = 11.2 mm, focal length = 60 mm, center frequency = 10 MHz; Olympus NDT, Waltham, MA, USA) was confocally mounted at the center opening (diameter = 11.2 mm) of the FUS transducer (Vlachos et al. 2010). A piece of polyurethane membrane (Trojan, Church & Dwight, Princeton, NJ, USA) was used to seal the transducer cone, which was filled with de-ionized and de-gassed water. The transducer system was attached to a computer-controlled 3-D positioner (Velmex, Lachine, QC, Canada). The FUS transducer was connected to a matching circuit and driven by a computer-controlled function generator (Agilent, Palo Alto, CA, USA) and a 50-dB power amplifier