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● *Original Contribution*

IDENTIFYING THE INERTIAL CAVITATION THRESHOLD AND SKULL EFFECTS IN A VESSEL PHANTOM USING FOCUSED ULTRASOUND AND MICROBUBBLES

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Abstract—Focused ultrasound (FUS) in combination with microbubbles has been shown capable of delivering large molecules to the brain parenchyma through opening of the blood-brain barrier (BBB). However, the mechanism behind the opening remains unknown. To investigate the pressure threshold for inertial cavitation of preformed microbubbles during sonication, passive cavitation detection in conjunction with B-mode imaging was used. A cerebral vessel was simulated by generating a cylindrical hole of 610 μm in diameter inside a polyacrylamide gel and saturating its volume with microbubbles. Definity microbubbles (Mean diameter range: 1.1-3.3 μm, Lantheus Medical Imaging, N. Billerica, MA, USA) were injected prior to sonication (frequency: 1.525 MHz; pulse length: 100 cycles; PRF: 10 Hz; sonication duration: 2 s) through an excised mouse skull. The acoustic emissions due to the cavitation response were passively detected using a cylindrically focused hydrophone, confocal with the FUS transducer and a linear-array transducer with the field of view perpendicular to the FUS beam. The broadband spectral response acquired at the passive cavitation detector (PCD) and the B-mode images identified the occurrence and location of the inertial cavitation, respectively. Findings indicated that the peak-rarefactional pressure threshold was approximately equal to 0.45 MPa, with or without the skull present. Mouse skulls did not affect the threshold of inertial cavitation but resulted in a lower inertial cavitation dose. The broadband response could be captured through the murine skull, so the same PCD set-up can be used in future *in vivo* applications. (E-mail: ek2191@columbia.edu) **© 2010 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Blood-brain barrier, BBB, Cavitation, Microbubble, Skull, Inertial, Vessel, Pressure.**

INTRODUCTION

It has been demonstrated that focused ultrasound (FUS) combined with microbubbles can open the blood-brain barrier (BBB) *in vivo* at acoustic pressures and duty cycles low enough that significant thermal effects may be avoided (Hynynen et al. 2001; Choi et al. 2007b). Neuronal damage throughout the sonicated region, as analyzed in histology, was not observed at acoustic pressures close to the threshold of BBB opening (McDannold et al. 2005). Different ultrasound contrast agents (UCA) and acoustic parameters such as frequency, pulse repetition frequency (PRF) and burst length have also been investigated. The pressure threshold of BBB opening was shown to decrease with burst length while the PRF did not affect the threshold (McDannold et al. 2008b). The mechanical index (MI) may also be an important indicator for predicting BBB opening (McDannold et al. 2008a). The acoustic pressure threshold for BBB opening was shown to increase with the applied sonication frequency. However, the threshold remained constant compared with the mechanical index.

The mechanism of BBB opening remains largely uncovered. Not only is the interaction between the acoustically driven bubble and brain capillaries unknown but also the effect of the skull on the BBB opening threshold has not been thoroughly described. Several studies investigating BBB opening require craniotomy (Hynynen et al. 2001; McDannold et al. 2005, 2006, 2007, 2008b). However, craniotomy is a difficult and time-consuming process that is associated with brain exposure, morbidity and occasional mortality. As a result, a lower frequency (260 kHz) has been proposed, which resulted in lower aberration through the skull when opening the BBB transcranially (Hynynen et al. 2005, 2006). Our group has characterized the FUS beam through the mouse skull in

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simulations and *ex vivo* skull experiments to study the effects of aberration and attenuation through the skull (Choi et al. 2007a). Localized, transcranial BBB opening in the murine hippocampus has also been reported (Choi et al. 2007b; Choi et al. 2010a; 2010b).

Acoustic cavitation, which refers to acoustically driven bubble activity, is considered to be the main cause for inducing BBB opening since it does not occur without injecting preformed microbubbles at a given acoustic setting. At low acoustic pressures, acoustically driven UCA size oscillations were shown to increase the permeability of surrounding cell membranes (van Wamel et al. 2004). At high acoustic pressures, inertial cavitation, *i.e.*, the collapse of bubbles, releases high energy and may create high temperatures, high pressures, and high velocity jets that may damage the surrounding structures (Miller et al. 1996). Therefore, knowing the pressure threshold for inducing inertial cavitation is important for controlling the effects induced by acoustically driven microbubbles. Apfel and Holland calculated the pressure threshold of inertial cavitation in water and showed that it increased with frequency (Apfel and Holland 1991). Most studies on the threshold of cavitation effects with UCA were based on the assumption of a free microbubble *i.e.*, not contained in a vessel phantom (Chomas et al. 2001a, 2001b; Giesecke and Hynynen 2003; Chen et al. 2003a, 2003b; Ammi et al. 2006). However, containment of the bubble within a vessel alters its behavior. Using theoretical and experimental evaluation in gel phantoms, Sassaroli and Hynynen reported that the threshold of inertial cavitation would be higher as the vessel diameter decreased as long as the diameter remained under 300 microns (Sassaroli and Hynynen 2006, 2007). Qin and Ferrara simulated the interaction between acoustically driven microbubbles in compliant and rigid microvessels and found that the threshold of bubble fragmentation was higher within rigid vessels when compared with compliant vessels (Qin and Ferrara 2006).

The relationship between acoustic cavitation and BBB disruption was previously investigated using a ring-shaped passive cavitation detector (PCD) surrounding a sonication transducer (McDannold et al. 2005, 2006). The peak-rarefactional pressure threshold of BBB opening and inertial cavitation at 260 kHz was found to be 0.29 MPa and 0.40 MPa, respectively, which suggested that inertial cavitation might not be necessary for BBB opening. However, the studies were performed following craniotomy and ignored any effects that the skull may introduce such as a change in the threshold of inertial cavitation.

The purpose of this paper was to investigate the effects of the mouse skull on the peak-rarefactional pressure threshold of inertial cavitation in a vessel phantom. *Ex vivo* mouse skulls were placed on the phantom to study transcranial wave propagation. The occurrence of inertial cavitation was investigated using simultaneous PCD and B-mode imaging. We also determined whether the cavitation response changes when the same acoustic pressures were applied at the presence or absence of the murine skull. At each pressure, different function generator voltages were applied to obtain the same acoustic pressure both in the presence and absence of the skull. The inertial cavitation dose (ICD) was used to identify the threshold of inertial cavitation and quantify the broadband response.

MATERIALS AND METHODS

Experimental set-up

The experimental set-up is shown in Figure 1. A single-element circular focused ultrasound transducer (Riverside Research Institute, New York, NY, USA) was driven by a function generator (Agilent Technologies, Palo Alto, CA, USA) through a 50-dB power amplifier (ENI Inc., Rochester, NY, USA). The center frequency, focal depth, outer radius and inner radius of the FUS transducer were 1.525 MHz, 90 mm, 30 mm and 11.2 mm, respectively. A single-element diagnostic transducer (center frequency: 7.5 MHz, focal length: 60 mm), which was driven by a pulser-receiver (Panametrics, Waltham, MA, USA), was positioned through the opening of the FUS transducer. These two transducers were confocally aligned. A cone filled with degassed and distilled water was attached to the transducer assembly. The transducer was then mounted on a computer-controlled positioner (Velmex Inc., Bloomfield, NY, USA). The dimensions of the focal region were measured and a lateral and axial full-width at half maximum (FWHM) intensity were of approximately 1.32 and 13.0 mm, respectively.

A 5-cm broadband, cylindrically focused hydrophone (Sonic Concepts, Bothell, WA, USA) with a cylindrical focal region (height 19 mm, diameter 3.64 mm) was placed at 60° (60°-PCD, Fig. 1a) or 90° (90°-PCD, Fig. 1b) from the longitudinal axis of the FUS beam. The hydrophone holder was adjusted to confocally align the hydrophone and the FUS transducer. The acoustic emissions from the microbubbles were acquired by the hydrophone followed by a 20-dB amplification (model 5800; Olympus NDT, Waltham, MA, USA) and collected using a digitizer (model 14200; Gage Applied Technologies, Inc., Lachine, QC, Canada).

Each sonication set included a pulse length of 100 cycles (67 μs) and a pulse repetition frequency (PRF) of 10 Hz. The total sonication duration of a sonication set was 2 s, *i.e.*, 20 pulses. Acoustic signals emitted from microbubbles were acquired for each pulse. The peak-rarefactional pressure amplitude ranged between 0.30 and 0.90 MPa at a 0.15 MPa step size as calibrated in our previous studies (Choi et al. 2007b).

The vessel phantom was constructed using acrylamide following Takegami et al. without the egg protein