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Feasibility of noninvasive cavitation-guided blood-brain barrier opening using focused ultrasound and microbubbles in nonhuman primates

Yao-Sheng Tung,1 Fabrice Marquet,1 Tobias Teichert,2 Vincent Ferrera,2 and Elisa E. Konofagou1,3,a
1Department of Biomedical Engineering, Columbia University, New York, New York 10027 USA
2Department of Neuroscience, Columbia University, New York, New York 10032 USA
3Department of Radiology, Columbia University, New York, New York 10032 USA

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In vivo transcranial and noninvasive cavitation detection with blood-brain barrier (BBB) opening in nonhuman primates is hereby reported. The BBB in monkeys was opened transcranially using focused ultrasound (FUS) in conjunction with microbubbles. A passive cavitation detector, confocal with the FUS transducer, was used to identify and monitor the bubble behavior. During sonication, the cavitation spectrum, which was found to be region-, pressure-, and bubble-dependent, provided real-time feedback regarding the opening occurrence and its properties. These findings demonstrate feasibility of transcranial, cavitation-guided BBB opening using FUS and microbubbles in noninvasive human applications. © 2011 American Institute of Physics.

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Most neurological disorders and neurodegenerative diseases, including Alzheimer’s and Parkinson’s, remain difficult to treat because of the impermeability of the blood-brain barrier (BBB). Mechanical stress induced by the activation of microbubbles in an acoustic field is currently the only noninvasive approach to temporar
dy open the BBB, without damaging the surrounding tissue.1,2 To date, BBB opening with focused ultrasound (FUS) has been achieved in different animals, including mice,2 rabbits,3 rats,4 and pigs.5 So far, this method has not been used in primates. It is nontrivial to extend this method to new species due to differences in physiology and anatomy.

It has been shown recently that a passive cavitation detector (PCD) can be used to transcranially acquire the acoustic emissions stemming from the interaction between the microbubble and the brain tissue during BBB opening in mice.5,7 The feasibility of transcranial cavitation detection in monkeys, however, remains unknown since the thickness and attenuation of the monkey skull is around 2.5 times higher than the murine skull.8 We have recently also demonstrated that bubble size plays an important role in BBB opening.9 Both monodispersed and polydispersed microbubbles are used in this study in order to confirm the effect of bubble size in nonhuman primates.

The experimental setup is shown in Fig. 1. A single-element, circular FUS transducer with a hole in its center 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 500 kHz, 90 mm, 30 mm, and 11.2 mm, respectively. A single-element PCD (center frequency: 7.5 MHz, focal length: 60 mm, Olympus NDT; Waltham, MA, USA) through the center hole of the FUS transducer. The two transducers were aligned with the later. The PCD was connected to a digitizer (Gage Applied Technologies, Inc., Lachine, QC, Canada) through a 20 dB preamplifier (5800, Olympus NDT; Waltham, MA, USA), and used to passively acquire acoustic emissions from microbubbles.

The experiment was performed on three (n=3) male macaque monkeys with three protocols shown in Table I. The targeting procedure has been described elsewhere by Marquet et al.10 In the case of monkey 1, the 4–5 μm microbubbles were manufactured in-house and size-isolated using differential centrifugation.11 Polydispersed Definity® microbubbles (Lantheus Medical Imaging, MA, USA) were used in the remaining two animals. The sonication was performed immediately after intravenous (IV) injection of 500 μL microbubbles for all monkeys. All methods were approved by the Institutional Animal Care and Use Committee at Columbia University and the New York State Psychiatric Institute.

Magnetic resonance imaging (MRI) at 3.0 T (Philips Medical Systems, Andover, MA, USA) was used to confirm

FIG. 1. (Color online) Experimental setup of in vivo FUS-induced BBB opening and transcranial cavitation detection in the monkey. A 7.5 MHz pulse-echo transducer served as the PCD.

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aAuthor to whom correspondence should be addressed. Present address: Dr. Elisa E. Konofagou, Department of Biomedical Engineering, Columbia University 351 Engineering Terrace, mail code 8904 1210 Amsterdam Avenue, New York, NY 10027 USA. Electronic mail: ek2191@columbia.edu. Tel.: 212-854-9661/212-342-0863. FAX: 212-342-5773.
TABLE I. Three protocols applied in this study.

<table>
<thead>
<tr>
<th></th>
<th>Monkey 1</th>
<th>Monkey 2</th>
<th>Monkey 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-N pressure (MPa)</td>
<td>0.30, 0.45</td>
<td>0.20, 0.25, 0.30</td>
<td>0.30, 0.45</td>
</tr>
<tr>
<td>Pulse length (cycles)</td>
<td>5000</td>
<td>100</td>
<td>5000</td>
</tr>
<tr>
<td>Microbubble</td>
<td>4–5 μm</td>
<td>Definity</td>
<td>Definity</td>
</tr>
<tr>
<td>PRF (Hz)</td>
<td>2</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Sampling rate (MHz)</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Focus (below skull)</td>
<td>4 cm</td>
<td>1 cm</td>
<td>4 cm</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>14</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>

and quantify the BBB opening following the opening. A three-dimensional (3D) spoiled gradient T$_1$-weighted sequence (TR/TE=20/1.4 ms; flip angle: 30°; NEX=2; spatial resolution: 500×500 μm$^2$; slice thickness: 1 mm with no interslice gap) was applied after intravenous (IV) injection of gadodiamide (Omniscan®, GE Healthcare, Princeton, NJ, USA). MR imaging was performed 1 h after sonication to confirm the location of the BBB opening. A time-frequency map of the acoustic emission was generated using a customized spectrogram function [eight cycles, i.e., 16 μs, Chebyshev window; 98% overlap; 4096-point Fast Fourier Transform (FFT)] in MATLAB$^\text{®}$ (2010a, Mathworks, Natick, MA).

The MR images and the corresponding spectrogram of the first pulse for monkey 1 are depicted in Fig. 2. As a result of the deposition of the MRI contrast agent in the brain tissue after ultrasound exposure, the MR images indicated that the BBB was opened at 0.30 MPa [Figs. 2(d), 2(e), and 2(g)] and 0.45 MPa [Figs. 2(f) and 2(h)] for the 4–5 μm bubbles. The corresponding spectrogram [Figs. 2(b) and 2(c)] showed that the broadband response, i.e., the inertial cavitation, occurred at 0.30 and 0.45 MPa. No harmonics were present in the spectrogram at 0.30 MPa without microbubble administration [Fig. 2(a)], which confirmed our previous findings in mice. The spectrogram can also be used to determine the position of the focus. The white arrow in Fig. 2(c) indicates the time-point of occurrence of the second harmonic coincides with the travel distance to the skull.

FIG. 2. (Color online) The BBB opening in Monkey 1 confirmed by 3D-MRI images. No higher harmonics or broadband response are detected at 0.30 MPa in (a) the spectrogram without microbubble administration. The corresponding spectrogram of the first pulse with microbubbles administration shows that the broadband acoustic emissions are detected at (b) 0.30 MPa and (c) 0.45 MPa. The 3D-MR images confirm that the BBB is opened at (d), (e), and (g) 0.30 MPa and (f) and (h) 0.45 MPa with inertial cavitation. The yellow box in the sagittal plane in (d) defines a region of interest from which images in (e) and (f) were acquired. The coronal plane with BBB opening is provided at (g) 0.30 MPa and (h) 0.45 MPa. The vertical white arrow in (c) indicates that the time-point of occurrence of the second harmonic coincides with the travel distance to the skull.

FIG. 3. (Color online) (a) The spectrogram without microbubble administration shows that all the harmonics and broadband response are from microbubbles. Spectrograms during PUS sonication with monkey 2 at (b) 0.20 MPa, (c) 0.25 MPa, (d) 0.30 MPa, and MR images with (e) coronal and (f) sagittal planes show that the broadband response is detected at all pressure but no BBB opening is induced (yellow circle).
that the time-point of occurrence of the second harmonic coincides with the travel distance to the skull. Therefore, harmonics higher than the third harmonic and any broadband response are due to microbubble effects [Figs. 2(b) and 2(c)].

Figure 2 shows that the BBB opening and the transcranial detection of cavitation spectra in monkeys are both feasible and that the cavitation spectra may differ among brain regions. The intensities of MRI contrast enhancement in the BBB opening region at 0.30 MPa and 0.45 MPa were 96.71 and 42.07, respectively. These differences may be due to a higher concentration of microbubbles in the specific sonicated region at 0.30 MPa. This would explain both the enhanced MRI contrast and the stronger broadband response.

In this study, the BBB was not opened in all cases of inertial cavitation. In the case of monkey 2, given that the medial areas were targeted, the focus included the superior sagittal sinus that, due to the large volume of microbubbles circulating, resulted in a larger amplitude of the cavitation spectrum (Fig. 3). Measuring the cavitation spectrum may, therefore, be helpful to determine whether a large vessel is in the path of the FUS beam and thus predict or avoid its effects on inducing BBB opening. The exact location of the focus in the brain is difficult to predict; second, the exact location of large vessels in the brain relative to the beam is not known a priori. Hence, the relationship between the amplitude of the cavitation spectrum, the area of BBB opening, and the BBB opening threshold will provide valuable additional information regarding the presence of large vessels close to the focus. This information can thus be used to (1) predict whether opening of the BBB was obstructed due to the focal spot proximity to a large vessel and subsequent shielding and (2) adjust the targeting accordingly to achieve BBB opening, i.e., avoid shielding by large vessels.

Monkeys 1 and 3 differed only with respect to the size of the microbubbles. BBB opening was only observed with the 4–5 μm microbubbles, not the Definity microbubbles; hence, the relationship between the amplitude of the cavitation spectrum, the area of BBB opening, and the BBB opening threshold will provide valuable additional information regarding the presence of large vessels close to the focus. This information can thus be used to (1) predict whether opening of the BBB was obstructed due to the focal spot proximity to a large vessel and subsequent shielding and (2) adjust the targeting accordingly to achieve BBB opening, i.e., avoid shielding by large vessels.

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In conclusion, the noninvasive and transcranial cavitation detection during BBB opening in nonhuman primates was shown in this study. In addition, the MRI contrast enhancement and cavitation response were shown to be region and/or microbubble-size dependent. Inertial cavitation may not induce BBB opening when the focus overlaps with large vessels such as the superior sagittal sinus. Therefore, this technique might be used for a cavitation-guided BBB opening to better monitor the target of sonication. Further studies will be performed to optimize the application in primates and determine the correlation between the location of BBB opening and the cavitation spectrum.

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FIG. 4. (Color online) (a) Spectrogram without microbubble administration shows that all the harmonics and broadband response correspond microbubbles. (b) Spectrogram at 0.45 MPa shows ultraharmonic and broadband response occurred. The MR image with (c) sagittal plane, however, shows that no BBB opening is induced in the sonicated region (yellow circle).