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● *Original Contribution*

SAFETY VALIDATION OF REPEATED BLOOD–BRAIN BARRIER DISRUPTION USING FOCUSED ULTRASOUND

THIELE KOBUS,*† NATALIA VYKHODTSEVA,* MAGDALINI PILATOU,* YONGZHI ZHANG,* and NATHAN MCDANNOLD*

*Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; and †Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

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Abstract—The purpose of this study was to investigate the effects on the brain of multiple sessions of blood–brain barrier (BBB) disruption using focused ultrasound (FUS) in combination with micro-bubbles over a range of acoustic exposure levels. Six weekly sessions of FUS, using acoustical pressures between 0.66 and 0.80 MPa, were performed under magnetic resonance guidance. The success and degree of BBB disruption was estimated by signal enhancement of post-contrast T1-weighted imaging of the treated area. Histopathological analysis was performed after the last treatment. The consequences of repeated BBB disruption varied from no indications of vascular damage to signs of micro-hemorrhages, macrophage infiltration, micro-scar formations and cystic cavities. The signal enhancement on the contrast-enhanced T1-weighted imaging had limited value for predicting small-vessel damage. T2-weighted imaging corresponded well with the effects on histopathology and could be used to study treatment effects over time. This study demonstrates that repeated BBB disruption by FUS can be performed with no or limited damage to the brain tissue. (E-mail: thiele.kobus@radboudumc.nl) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Focused ultrasound, Blood–brain barrier disruption, Multiple sessions, Safety study, Magnetic resonance guidance, Histologic evaluation.**

INTRODUCTION

The blood–brain barrier (BBB) is a functional and structural barrier that protects the brain. It regulates transport of molecules from the vasculature to the central nervous system (Abbott and Romero 1996). The BBB consists of endothelial cells connected by tight junctions, pericytes, a basement membrane and endfeet of astrocytes. Only small (molecular weight < 400 Da), hydrophobic molecules can pass the BBB. The BBB is a hurdle in the development of drugs effective in the central nervous system because practically all large-molecule drugs and more than 98% of small-molecule drugs do not pass the BBB (Pardridge 2003). Several approaches have been proposed to circumvent this barrier (*e.g.,* Bobo et al. 1994; Doolittle et al. 2000; Guerin et al. 2004; Pardridge 2002a, 2002b), but these are either invasive or non-localized. In clinical practice, many therapeutic agents need to be administered multiple times over the course of several weeks or months to be effective. This means that the BBB needs to be disrupted repeatedly over an extended period of time. Therefore, a method that can non-invasively and reversibly disrupt the BBB at targeted locations would have major impact on clinical neuroscience.

A technique with this potential was introduced in 2001 (Hynynen et al. 2001). The researchers used focused ultrasound (FUS) in combination with micro-bubbles circulating in the vasculature to temporarily disrupt the BBB. In the ultrasound focal region, an interaction between the micro-bubbles, small gas bubbles usually used as ultrasound contrast agent, and ultrasound waves takes place. Pre-clinical studies have shown that these interactions cause a temporary disassembly of the tight junction proteins and stimulate active transport, making it possible to deliver drugs through the BBB (Fan et al. 2011; Hynynen et al. 2001; Shang et al. 2011; Sheikov et al. 2006, 2008; Xia et al. 2012). A few hours after the focused ultrasound therapy, the barrier is closed, and the brains appear normal in light microscopy (Baseri et al. 2010; Hynynen et al. 2005; Hynynen et al. 2006; McDannold et al. 2005).

Address correspondence to: Thiele Kobus, 221 Longwood Avenue, Boston, MA 02115, USA. E-mail: thiele.kobus@radboudumc.nl

When the BBB is disrupted by FUS in the presence of micro-bubbles, small vessel damage can occur, which can have minimal to severe consequences. These consequences can be studied with histologic examinations. Previously, the histologic effects of a single treatment of FUS in combination with micro-bubbles have been examined (Baseri et al. 2010; Hynynen et al. 2005, 2006; McDannold et al. 2005). These studies have shown only negligible effects on the tissue, largely related to the presence of microscopic regions containing extravasated erythrocytes, so-called petechiae. Histologic effects of multiple sonications were investigated in one non-human primate, but were not studied systematically (McDannold et al. 2012). The cumulative histologic effects of repeated stress to the brain vasculature are unknown.

In this study, the effects of repeatedly disrupting the BBB were studied. By performing the sonications under magnetic resonance (MR) guidance, the ultrasound focus could be targeted at the same brain regions during six weekly treatments. Furthermore, MR imaging (MRI) was used to determine the success of each treatment and obtain information about the effects of the treatment over time, although at a relatively low spatial resolution. Histologic analysis was performed for each animal after the last session. MRI and histopathology provided complementary information about the effects of repeated BBB disruptions. The results of these experiments will be important to move this technology to the clinic and to aid in evaluating the potential risks and benefits for different therapeutic applications.

MATERIALS AND METHODS

Animals

The study was approved by the Institutional Animal Care Committee. Fifteen healthy Sprague-Dawley rats (Charles River Laboratories, Boston, MA) were included in this study. The animals were divided in three groups. Each group received six weekly ultrasound treatments at a different set of pressure amplitudes. The weight of the animals was measured each week. The animal weight at the start of the sonications was 325 ± 12 g for group 1 (lowest pressure group), 276 ± 63 g for group 2 and 197 ± 83 g for group 3 (highest pressure group). The animals were sacrificed between 1 h and 36 h after the last sonication.

MR-guided ultrasound procedures

Setup. The setup for the sonications is shown in Figure 1. A single-element, spherically-focused transducer (diameter = 10 cm, f-number = 0.8, frequency = 690 kHz) was used to generate the ultrasound field. The half-maximum pressure amplitude width and length of the focal region were 2.3 and 14 mm, respectively (Hynynen et al. 2005). The transducer was mounted to a three-axis positioning system, placed in a tank with degassed water and connected to a matching circuit. To generate the ultrasound signal, an arbitrary waveform generator (Model 395, Wavetek Inc., San Diego, CA) and an RF amplifier (Model 240 L, ENI Inc., Rochester, NY) were used. The electrical power was monitored with a power meter (Model E4419 B, Agilent, Santa Clara, CA) and a dual-directional coupler (Model C594810-C, Werlatone, Patterson, NY).

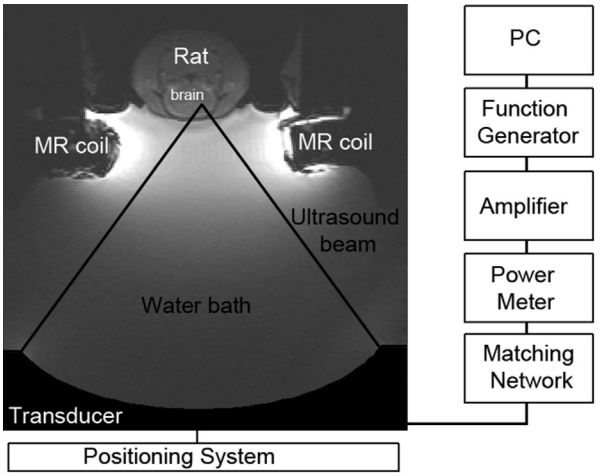

Fig. 1. The setup for the focused ultrasound treatments. The rat was placed in supine position in a holder on top of a water bath. The 690-kHz transducer was placed in the water bath and connected to a positioning system and matching network. To obtain the magnetic resonance images, a home-made transmit/receive surface coil was used.

Animal preparation. The animals were anesthetized with a mix of 80 mg/kg ketamine (Aveco Co., Inc., Fort Dodge, IA) and 10 mg/kg of xylazine (Lloyd Laboratories, Shenandoah, IA) *via* intra-peritoneal injection. The hair on the animal's head was removed and a catheter was inserted into the tail vein. The rat was placed in supine position in the sonication system (Fig. 1).

MRI guidance. The sonications were performed in a clinical 3 T MRI system (Signa, GE Healthcare, Milwaukee, WI). After placement of the animal, fast gradient echo images were obtained to localize the brain. Next, axial pre-sonication T1-weighted imaging (T1-WI) and, in most sessions, axial T2-weighted imaging (T2-WI) were obtained with a fast spin echo sequence. The image parameters are provided in Table 1. T1-WI in combination with a gadolinium-based MRI contrast agent is commonly used to confirm BBB disruption. The intact BBB does not allow these agents to extravasate from the capillaries. A hyperintense region on post-contrast T1-weighted images indicates extravasation of gadolinium and thus successful BBB disruption. Therefore, after the sonications, a bolus of 0.25 mL/kg MRI contrast agent gadopentetate