Ultrasound in Med. & Biol., Vol. 39, No. 8, pp. 1420–1428, 2013

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2013.03.006

● *Original Contribution*

CREATING BRAIN LESIONS WITH LOW-INTENSITY FOCUSED ULTRASOUND WITH MICROBUBBLES: A RAT STUDY AT HALF A MEGAHERTZ

YUEXI HUANG,* NATALIA I. VYKHODTSEVA,† and KULLERVO HYNYNEN*‡

*Sunnybrook Research Institute, Toronto, ON, Canada; †Department of Radiology, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA; and ‡Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

(*Received* 6 *July* 2012; *revised* 21 *February* 2013; *in final form* 5 *March* 2013)

Abstract—Low-intensity focused ultrasound was applied with microbubbles (Definity, Lantheus Medical Imaging, North Billerica, MA, USA; 0.02 mL/kg) to produce brain lesions in 50 rats at 558 kHz. Burst sonications (burst length: 10 ms; pulse repetition frequency: 1 Hz; total exposure: 5 min; acoustic power: 0.47–1.3 W) generated ischemic or hemorrhagic lesions at the focal volume revealed by both magnetic resonance imaging and histology. Shorter burst time (2 ms) or shorter sonication time (1 min) reduced the probability of lesion production. Longer pulses (200 ms, 500 ms and continuous wave) caused significant near-field damage. Using microbubbles with focused ultrasound significantly reduced acoustic power levels and, therefore, avoided skull heating issues and potentially can extend the treatable volume of transcranial focused ultrasound to brain tissues close to the skull. (E-mail: khynynen@sri.utoronto.ca) © **2013 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Focused ultrasound, Microbubble, Brain lesion, Hemorrhage, Transcranial ultrasound.**

INTRODUCTION

In the past decade, transcranial high-intensity focused ultrasound (HIFU) has emerged as a non-invasive neurosurgical tool for the potential treatment of various brain diseases. The hemispherical design of large-array transducers operating below 1 MHz allows the passage of acoustic energy through the maximum available skull area and, therefore, reduces skull heating while delivering sufficient energy to thermally ablate the focal volume (Sun and Hynynen 1999). The large array also enables phase correction on transducer elements to restore the focal distortion caused by variation in skull thickness and density (Clement and Hynynen 2002; Hynynen and Jolesz 1998; Hynynen and Sun 1999; Tanter et al. 1998). Furthermore, magnetic resonance imaging (MRI) provides excellent anatomic guidance and dynamic temperature monitoring of the focal spot and surrounding tissues (De Poorter et al. 1995; Ishihara et al. 1995), which significantly improves the accuracy of heat deposition and the safety profile of the procedure (Hynynen et al. 2004). Preliminary patient trials using a magnetic resonance-guided focused ultrasound (MRgFUS) brain system have been performed for tumor ablation (McDannold et al. 2010a) and neuropathic pain treatment (Jeanmonod et al. 2012; Martin et al. 2009).

Despite significant progress, thermal ablation is feasible only in the middle of the brain. If targets are close to the skull, near-field or far-field heating on that piece of bone may still be significant (McDannold et al. 2010b; Pulkkinen et al. 2011). Therefore, alternative methods have been explored to potentiate the ultrasound damage to the tissue with reduced acoustic intensity to avoid bone overheating. Previously it has been shown that an intravenous injection of preformed microbubbles reduces the focused ultrasound (FUS) power needed to produce brain lesions by an order of magnitude (McDannold et al. 2006a; Vykhodtseva et al. 2006). These studies were performed with a 1.5-MHz focused transducer with continuous wave (CW) sonication or with 50% duty cycles. Craniotomies were performed on rabbits to avoid acoustic attenuation. Significant temperature elevations were observed at the focal volume. However, the temperature increase was lower than the level required for thermal tissue ablation (5.9°C temperature elevation at 50% probability of tissue necrosis). Therefore, the presence of microbubbles during sonication might sensitize

Address correspondence to: Kullervo Hynynen, Imaging Research, Room S665 B, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, ON, M4N 3M5, Canada. E-mail: khynynen@sri.utoronto.ca

the tissue to thermal exposure. The details of the mechanism are still not well understood.

Microbubbles have also been used with FUS to reversibly disrupt the blood-brain barrier (BBB) and, hence, delivery of drugs to the brain (Choi et al. 2007; Hynynen et al. 2001; Liu et al. 2010; McDannold et al. 2008a, 2008b; Tung et al. 2011). Microbubbles act as a nucleation promotion agent in an ultrasound field for either stable or inertial cavitation, which has a mechanical impact on the microvasculature (McDannold et al. 2006b; Tung et al. 2010). With the proper combination of FUS parameters, it has been shown that the tight junctions between endothelial cells in brain capillaries can be reversibly broken up with no or only minimal red blood cell extravasation (Sheikov et al. 2004). So far, all these studies have been aimed at improving vascular permeability for drug delivery. Extensive hemorrhage/neuron damage was considered a side effect that should be avoided. To our knowledge, other than the two brain lesion studies of McDannold et al. (2006a) and Vykhodtseva et al. (2006), there are no published studies that have attempted to cause brain lesions by combining thermal exposure with FUS and microbubbles.

The previous studies on brain lesioning were performed at 1.5 MHz with craniotomies. In the present study, a 558-kHz FUS transducer was used, as this frequency is more suitable for transcranial sonication for human applications. The clinical brain HIFU system (ExAblate 4000, InSightec, Tirat Carmel, Israel) is available at 650 kHz and 230 kHz. Therefore, the results at 558 kHz are within the range to be translated for potential clinical applications. Continuous wave/long-duty-cycle sonications have been shown to cause near-field heating/tissue damage in the presence of microbubbles (McDannold et al. 2006a; Tung et al. 2006). In this study, short pulses similar to those used in BBB studies were applied, whereas acoustic parameters were increased to maximize the microvascular damage. The hypothesis was that with acoustic power higher than and/or sonication time longer than that used for BBB disruption, the capillary endothelial walls could be damaged, which would lead to disturbance of the blood supply and the formation of localized brain lesions.

METHODS

Animal preparation

Animal experiments were performed on 50 Sprague-Dawley rats weighing 400–600 g. The procedures were approved by the Institutional Animal Care Committee. Animals underwent anesthesia using a mixture of 50 mg/kg ketamine hydrochloride (Abbott Laboratories, North Chicago, IL, USA) and 10 mg/kg xylazine (Phoenix Pharmaceuticals, St. Joseph, MO, USA). Hair was removed from rat heads using hair clippers and depilatory lotion. A catheter was inserted into the tail vein for microbubble and MRI contrast agent injections.

Focused ultrasound

A MR-compatible focused ultrasound system was used with a 1.5-T MRI scanner (Signa, GE Healthcare, Milwaukee, WI, USA). The ultrasound beam was generated by a piezoelectric transducer (10-cm diameter, 7.8-cm radius of curvature, 558-kHz center frequency, manufactured in house). The focal volume was 3 mm in the lateral dimension and 10 mm in the axial dimension. The transducer was positioned in a degassed water tank using a MRI-compatible three-axis motorized system (Chopra et al. 2009). The experimental setup is illustrated with an annotated MR image (Fig. 1). The transducer was driven by a function generator (Model 395, Wavetek, San Diego, CA, USA) and radiofrequency amplifier (ENI, model 240 L, 50 dB, ENI, Rochester, NY, USA). The transducer's electrical impedance was matched to the output impedance of the amplifier (50 Ω) with a custom-made passive matching circuit. Electrical power was measured in CW mode by a power meter (Moldel 438A, Hewlett-Packard, Palo Alto, CA, USA) connected to a dual-directional coupler (Model C173, Werlatone, Brewster, NY, USA). Before the experiment, the power meter readings were calibrated using a radiation force method for acoustic power. Burst sonications at four acoustic power levels (0.47, 0.59, 0.89 and 1.3 W) were used. The peak pressure at the focus was calibrated using a factory-calibrated fiber-optic hydrophone (active tip

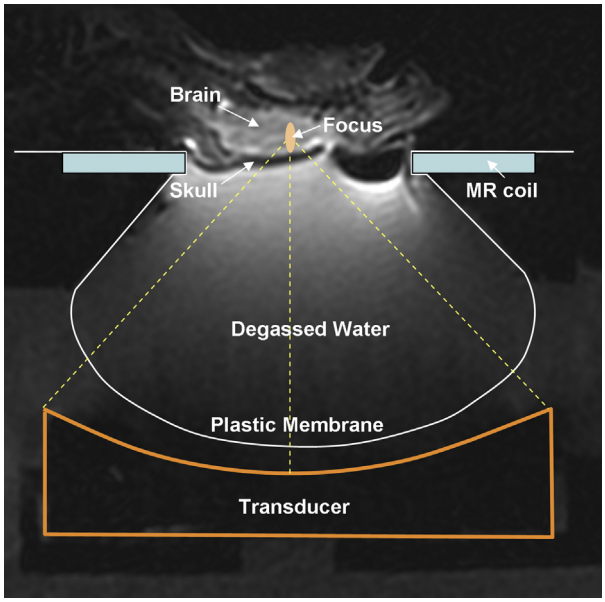

Fig. 1. Annotated magnetic resonance (MR) image illustrating the experimental setup.