Non-invasive generation and monitoring of ultrasound-induced blood-brain barrier opening in the murine hippocampus

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Introduction and Background – One of the difficulties in treating neurological diseases is the fact that neurologically potent drugs cannot be delivered to affected tissue regions because of the distinct brain vasculature, the blood-brain barrier (BBB), which regulates the flow of molecules between the blood vessels and the brain [1]. In the case of neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease, only a specific region of the brain, the hippocampus, is affected. To address this, we have used Focused Ultrasound (FUS), which has been shown to produce reversible and localized BBB opening when applied in the presence of gas-filled microbubbles [2, 3], to open the BBB in the murine hippocampus. In FUS, ultrasonic energy can be focused to a 0.5 to 2 mm region and up to 20 cm in depth. The purpose of this study was to understand the nature of the FUS-induced BBB opening *in vivo* in mice in the hippocampus region using high-resolution MRI. FUS was applied entirely noninvasively through the intact skull and skin and targeted a specific brain region. The hippocampus was targeted, mainly because we have long-term interest in studying the treatment of Alzheimer's disease, which affects this region. In addition, the *in vivo* dependence of microbubble concentration, FUS power, and vessel type distribution of the targeted brain region from FUS-induced BBB opening was investigated.

Materials and Methods - A therapeutic FUS transducer (1.525MHz) was confocally combined with a 7.5MHz diagnostic transducer that allowed for consistent, high precision targeting of the hippocampus [3]. To induce BBB opening, mice (n=4) were intravenously injected with microbubbles (Optison™; 25-50μL). The left hippocampus of their brains were then separately sonicated (pressure amplitudes: 0.53-0.80MPa; burst length: 20ms; duty cycle: 20%; duration: 2-4 shots, 30s per shot, 30s delay between shots) through their intact skull and skin. The right hippocampus was not targeted and acted as a control. Approximately one hour after sonication, the mice were injected intraperitoneally (IP) with a gadolinium-based MRI contrast agent (Omniscan™; 0.5mL) [4] and scanned with high-resolution sequential T1-weighted MR Imaging (9.4Tesla; Repetition Time / Echo Time: 246.1ms / 10ms; Bandwidth: 50,505.1Hz; Matrix Size: 256x256; Field of View: 1.92x1.92cm; Slice Thickness: 0.6mm; Number of Excitations: 5). The contrast-enhanced variation was monitored for a period of 120 min post-gadolinium injection (total number of MR images obtained: 28) to assess the time course of BBB opening. 11x11 pixel areas in the targeted (left) and control (right) hippocampus regions were selected and their intensities were averaged for each MR image. Any pixel intensity on the MRI that was above 2.5 standard deviations of the averaged control hippocampus region was determined to have undergone BBB opening. The approximate area of opening was then calculated by counting the pixels above that threshold [3].

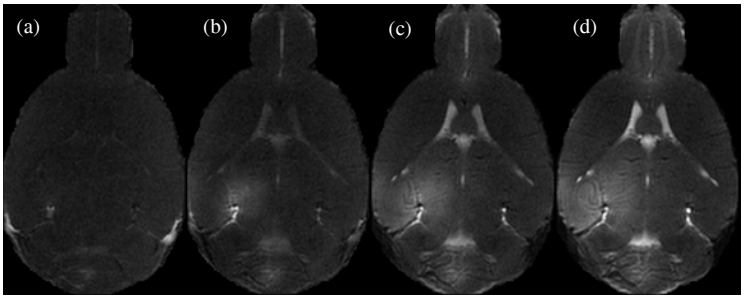

Figure 1 - T1 MRI scans of horizontal slices of a single mouse brain approximately 3mm beneath the top of the mouse skull . Images were obtained after injection of 25μL of Optison™ and sonication with a pressure amplitude of 0.80MPa. The ultrasound beam was focused onto the left hippocampus while the right hippocampus was not targeted as acted as a control. The images depict the slow diffusion of gadolinium (a) 17min, (b) 44min, (c) 82min, and (d) 120min after gadolinium injection.

Results - The threshold of BBB opening with an injection of 50μL of Optison was between 0.53 and 0.66MPa with the area of BBB opening increasing linearly with peak rarefractional pressure (data not shown). Reducing the amount of Optison™ injected to 25μL decreased the area of BBB opening by a three-fold (data not shown). Sequential MRI scans were also able to determine the influence of vessel density and size within the beam on the BBB opening. Using the optimized sonication parameters to induce consistent BBB opening (pressure: 0.8MPa; Optison™: 25μL), the path of the MRI contrast agent diffusion throughout the BBB-opened hippocampus region could be tracked over the course of two hours (Fig. 1). The posterior cerebral artery (PCA), because of its larger vessel size (and, therefore, higher Optison concentration interacting with the FUS beam), depicted a greater pixel intensity increase on the T1-weighted MR image at the sonicated region compared to the non-sonicated (control) and homogeneous brain tissue regions (Fig. 2). In addition, contrast-enhancement was first observed in the left PCA before spreading throughout the entire hippocampus region.

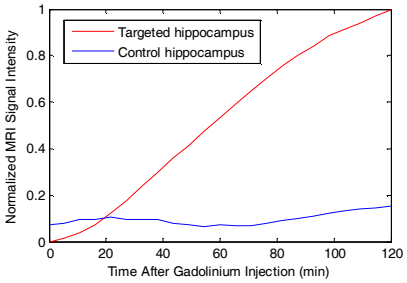

Figure 2 - Temporal analysis of gadolinium diffusion throughout the targeted (red) and control (blue) hippocampus region of a mouse brain after injection of 25μL Optison™ and sonication at 0.80MPa.

Conclusion - These findings demonstrate that BBB opening can be noninvasively, locally, and transcranially induced in the entire hippocampus of mice using FUS and microbubbles. In addition, the importance of microbubble concentration and vessel characteristics on opening the BBB in the mice has been determined. Finally, the use of optimized parameters for opening at the threshold, minimizing probability of irreversible damage, together with slow-diffusion MRI imaging, allowed for temporal analysis of the BBB opening. The MRI data revealed not only detection of the opening, but also the path which the model drug diffused through. We expect further optimization of this technique to be very useful in testing novel therapies in mice models for a variety of neurological pathologies such as Alzheimer's disease.

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