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Analysis of focused ultrasound-induced blood–brain barrier permeability in a mouse model of Alzheimer's disease using two-photon microscopy

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ABSTRACT

Transcranial focused ultrasound (FUS) can cause temporary, localized increases in blood–brain barrier (BBB) permeability for effective drug delivery to the brain. In pre-clinical models of Alzheimer's disease, FUS has successfully been used to deliver therapeutic agents and endogenous therapeutic molecules to the brain leading to plaque reduction and improved behavior. However, prior to moving to clinic, questions regarding how the compromised vasculature in Alzheimer's disease responds to FUS need to be addressed. Here, we used two-photon microscopy to study changes in FUS-mediated BBB permeability in transgenic (TgCRND8) mice and their non-transgenic littermates. A custom-built ultrasound transducer was attached to the skull, covering a cranial window. Methoxy-X04 was used to visualize amyloid deposits *in vivo*. Fluorescent intravascular dyes were used to identify leakage from the vasculature after the application of FUS. Dye leakage occurred in both transgenic and non-transgenic mice at similar acoustic pressures but exhibited different leakage kinetics. Calculation of the permeability constant demonstrated that the vasculature in the transgenic mice was much less permeable after FUS than the non-transgenic littermates. Further analysis demonstrated that the change in vessel diameter following FUS was lessened in amyloid coated vessels. These data suggest that changes in vessel diameter may be directly related to permeability and the presence of amyloid plaque may reduce the permeability of a vessel after FUS. This study indicates that the FUS parameters used for the delivery of therapeutic agents to the brain may need to be adjusted for application in Alzheimer's disease.

1. Introduction

Transcranial focused ultrasound (FUS) in conjunction with microbubble contrast agent has been shown to temporarily increase the permeability of the blood–brain barrier (BBB) and facilitate targeted drug delivery to the brain [1]. In preclinical models of Alzheimer's disease, antibodies against amyloid-β peptides have been delivered across the BBB using FUS [2,3]. Quantification of plaque pathology in the cortex demonstrated that FUS-mediated antibody delivery reduced the size and number of amyloid plaques after 4 days [3]. More recently, it has been established that FUS also promotes the delivery of endogenous therapeutic molecules and activates endogenous repair mechanisms leading to improvement in pathology and behavior [4,5]. FUS, without exogenous drug delivery, caused a reduction in plaque number and size throughout the cortex [4] and hippocampus [5] and was accompanied by increased neuronal plasticity and improved spatial learning behavior [5].

Despite the preliminary success of FUS as a delivery method and stand-alone treatment for Alzheimer's disease, many questions regarding the effects of FUS in the brain affected by amyloid pathology still remain. Amyloid is known to accumulate in extraneuronal plaques however it has been reported that in the majority of clinical Alzheimer's disease cases, amyloid is also found deposited in the adventitia of small and mid-sized arteries, a pathology known as cerebral amyloid angiopathy [6]. Cerebral amyloid angiopathy has been shown to compromise the integrity of the BBB, disrupt cerebrovascular regulation and increase the susceptibility of the vessels to breakage [7,8]. The presence of cerebral amyloid angiopathy is a significant risk factor for cerebral hemorrhage in the Alzheimer's population [9].

Several studies have reported that when appropriate parameters are applied, FUS can temporarily open the BBB without any deleterious effects on the healthy brain [10–12]. However, the presence of cerebral amyloid angiopathy suggests that FUS may be more likely to cause permanent damage to the vessels and surrounding brain parenchyma compared to the healthy brain. Therefore, further investigation into

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the effects of FUS-mediated BBB permeability in the presence of amyloid pathology is required.

Here, we used two-photon microscopy to investigate in real time the differences between FUS-induced permeability of plaque-burdened vessels in a mouse model of Alzheimer's disease compared to healthy vessels in non-transgenic littermates. We also explore the difference in permeability between the plaque-coated vessels and the non-plaque coated vessels in the transgenic mice. Overall, our goal was to understand the impact of vascular amyloid on the FUS-induced changes in permeability such that the safety of FUS-induced BBB opening for Alzheimer's disease patients can be evaluated in the future.

2. Materials and methods

2.1. Animal preparation

Transgenic mice from the Centre for Research in Neurodegenerative disease (TgCRND8) and their non-transgenic littermates were bred and housed in the Sunnybrook Research Institute animal facility. TgCRND8 mice have a double mutation of the amyloid precursor protein 695 (KM670/671/NL and V717F) and develop amyloid pathology and cognitive deficits by 2–3 months of age [13,14]. The mice have been reported to develop cerebral amyloid angiopathy which increases with age. To ensure that sufficient quantities of vascular plaque were present in the brain, male and female mice at 6–8 months of age and their non-transgenic controls were used in this study ($n = 48$). A cranial window preparation was performed on each animal and immediately following the imaging and treatment session, the animals were sacrificed. All animal procedures were approved by the Animal Care Committee at Sunnybrook Research Institute and were in accordance with the guidelines established by the Canadian Council on Animal Care.

Methoxy-X04 was generously provided by Dr. William Klunk (University of Pittsburgh) for the visualization of amyloid plaques *in vivo*. Methoxy-X04 was administered intraperitoneally at a dose of 5–10 mg/kg, 24 h prior to the onset of surgery [15]. On the experiment day, animals were anesthetized with 5% isoflurane in an induction chamber and reduced to 2% for the remainder of the experiment. Physiology was monitored using a pulse oximeter and rectal temperature probe. A tail vein catheter was inserted for the administration of microbubbles and imaging reagents. The mice were positioned in a stereotaxic frame using ear and incisor bars, and a cranial window (3 mm diameter) was prepared over the right hemisphere, posterolateral to Bregma (Fig. 1A). The cranial window was filled with 1% agarose and an 8 mm coverslip was secured to the skull over the window using cyanoacrylate glue (Fig. 1B). The transducer and coverslip (10 mm diameter) were attached on top to facilitate FUS application [16]. The transducer also served as a well for the water immersion objective (Fig. 1B).

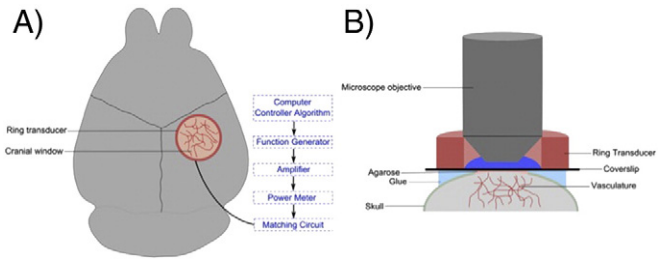

Fig. 1. Schematic of experimental setup. A) A cranial window was prepared in the mouse skull, posterolateral to Bregma. The transducer was glued over the window and connected with a matching circuit and the components required to drive the ultrasound. B) From a sagittal view, it can be observed that the ring transducer served as a well for the water immersion objective.

2.2. Two-photon imaging

The mice were secured on the stereotactic frame and the entire frame was moved to the microscope stage. Fluorescent dextran (70 kDa Texas Red, dissolved in PBS, Invitrogen) was administered through the tail vein to visualize the vasculature. Imaging was performed using the FV1000MPE two-photon laser scanning microscope (Olympus) with a mode locked Ti:Sapphire laser and 810 nm excitation wavelength (MaiTai, Spectraphysics). A 25× water-immersion objective lens (1.05 NA, 2 mm working distance, Olympus) was used to collect images. 10 XYZT stacks capturing lateral images of 512 × 512 pixels (1 μm resolution, 4–8 μs/pixel) were up to 300 μm below the cortical surface. Several XYZT stacks of the vascular were used to generate baseline images prior to the induction of BBB permeability.

2.3. FUS-induced BBB permeability

A single-element, ring-shaped piezoelectric ultrasound transducer (thickness: 1.4 mm, height: 1.19 mm, outer diameter: 10 mm, frequency: 1.15–1.30 MHz) was custom manufactured. The transducer was driven by a function generator (Agilent) and a 53-dB Radio Frequency Power Amplifier (NP Technologies Inc.). During sonication, the forward and reflected powers were monitored by a power meter built in-house and the entire sonication process was controlled by a custom computer software program. Microbubbles (perfluorobutane gas encapsulated by a 1,2-distearoyl-*sn*-glycero-3-phosphocholine with polyethylene glycol stearate shell) were prepared as previously described [17] and injected at a final concentration of 0.04 ml/kg prior to sonication. Sonications were carried out at 0.4–0.8 MPa estimated *in situ* pressures, 10 ms bursts and 1 Hz pulse repetition frequency for a total duration of 120 s.

Fluorescent dextran was injected intravenously followed by two-photon imaging of the naïve vasculature. Immediately following microbubble injection, the sonication began. This point served as time = 0. Two-photon imaging was continuous during sonication and lasted for 1 h after leakage had been observed.

2.4. Image processing and analysis

4D image stacks were visualized in Image J as maximum intensity projections along the z direction. Using an automatic vessel segmentation algorithm written in Matlab (The Mathworks, Natick, MA, USA) as previously reported [18], extravascular and intravascular compartments were separated and temporal fluorescent signal associated with each compartment ($I_i(t)$ and $I_e(t)$) was measured. Permeability coefficient, a measure of the exchange capacity between the two compartments, was deduced from $I_i(t)$ and $I_e(t)$ using the formulae introduced by Dreher et al., [19]:

$$P(t) = \frac{dI_e/dt}{\frac{I_i(t)}{1-HCT} - \frac{I_e(t)}{V_e/V_i}}$$

where V_e/V_i is the volume fraction between the two compartments, and HCT = 0.45 represents an average hematocrit level of all blood vessels within the imaging field of view. Based on the peak time of the permeability curve $P(t)$, onset of BBB permeability was determined.

Quantification of leakage dynamics and the measurement of vessel diameter were performed manually using Image J, by a blinded observer. With respect to the identification of fast or slow leakage, an instance of leakage was only quantified if the temporal and spatial origin of the leakage could clearly be identified. In some field of view, multiple leakage types could be distinguished and therefore were all included.