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Characterizing Focused Ultrasound-Induced Changes using 2-Photon Microscopy and MRI in a Mouse Model of Alzheimer's Disease.

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Synopsis

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Motivation: Focused ultrasound (FUS) with microbubbles is a promising technology for opening the blood-brain barrier (BBB) to deliver therapeutics to the brain. Answering fundamental questions about the process of BBB opening will aid in the translation of this method to the clinic.

Goal(s): Use FUS, 2-photon microscopy, MRI and immunohistochemistry to investigate FUS-induced changes in wild type and 5X-FAD AD mice.

Approach: A cross-sectional study involving MRI, 2PM, and IHC to examine BBB opening at multiple timepoints in a disease model.

Results: Results suggest BBB opening occurs in capillaries and that the BBB opening is similar between WT and 5X-FAD mice.

Impact: DCE imaging and sub-micron imaging of the microvasculature after BBB opening provide insight into which vessels are opened after FUS and improve pharmacokinetic understanding of the paravascular space in an Alzheimer's Disease model.

Introduction

Focused ultrasound (FUS) has been introduced as a novel method of transiently opening the blood-brain barrier (BBB) for drug delivery and therapy in neurodegenerative disease[1]. Previous work by this lab demonstrated that 2-photon microscopy can be used to visualize BBB disruption in the mouse cortex in-vivo[2]. The present study extends earlier work by evaluation of two questions: **1.** *Which vessels are opened in the cerebrovasculature after FUS?* **2.** *Does pathology in the 5X-FAD Alzheimer's disease mouse model alter BBB opening or closing?* To address these questions, multi-modal experiments were conducted using focused ultrasound, in-vivo 2-photon microscopy (2PM), T1-wieghted MRI using gadolinium-based contrast agent (GBCA), and dynamic contrast enhanced (DCE) MRI.

Methods

All experimental procedures were performed at the University of Arizona Translational Bioimaging Resource under approved IACUC protocols. To address the first question of which vessels open following FUS, 4 control and 4 FUS-treated C57BL/6 mice underwent a skull-thinning procedure[4] and 2PM experimental procedure following methods outlined in previous work[2] and in figure 1. Fluorescently-labeled dextran (Rhodamine, Ex/Em = 545/566 nm) was injected. A Z-stack (FOV = 400 x 400 um^2, 0.78 x 0.78 um^2 in-plane resolution, 1 um step size, 200 steps. Frame rate = 824.73 ms) was acquired before and after FUS. A 2D timeseries (FOV = 400 x 400 um^2, 1.57 x 1.57 um^2 resolution, 300 images, 306.59 ms per image) was acquired during an injection of fluorescently-labeled dextran (FITC, Ex/Em = 490/525 nm). A second 2D timeseries was collected at higher resolution for 5 minutes (FOV = 400 x 400 um^2, 0.78 x 0.78 um^2 resolution, 375 images, 824.73 ms/image). After the 2D time series, Z-stacks were acquired for 1 hour.
To answer the second question, a cross-sectional study was conducted involving 6 different mouse groups (4 mice per group, 24 mice total). C57/Bl6 Wild-Type and 5X-FAD mice received FUS treatment following previously published procedures[3]. After FUS, the mice were imaged in the Bruker BioSpec 7T preclinical MRI scanner. The experimental setup is outlined in figure 2. The imaging included coronal T1-weighted (T1W, TR/TEeffective = 600/8 ms, echo train length = 2, ESP = 8 ms, 16 averages, FOV = 12.8 mm x 25.6 mm x 12.6 mm, 200 x 200 um in-plane resolution, 0.6 mm slice thickness, 21 contiguous slices, scan time = 5:07 min:sec) and axial T1W (TR/TEeffective = 600/8 ms, echo train length = 2, ESP = 8 ms, 8 averages, FOV = 19.2 mm x 12.8 mm, 100 x 100 um in-plane resolution, 0.6 mm slice thickness, 21 contiguous slices, scan time = 5:07 min:sec) images, as well as a T1map (VTR, TR/TE = 867.5/7 ms, echo train length 4, ESP = 7 ms, FOV =15.0 x 15.0 mm x 1.5mm, 117 x 156 um in-plane resolution, 0.5mm slice thickness, 3 contiguous slices, scan time = 7:15 min:sec) and DCE (FLASH, TR/TE = 100/2 ms, flip angle = 60o, FOV =15.0 x 15.0 mm x 1.5mm, 117 x 156 um in-plane resolution, 0.5mm slice thickness, 3 contiguous slices, scan time = 20 min:sec). Mice were sacrificed either immediately after MRI, or after a second round of MR imaging at T = 48 hours or T = 2-weeks post-FUS. The mouse brains were extracted for future immunohistochemistry.

Results

Application of FUS resulted in fluorescent dye leakage into the paravascular space due to BBB opening within 5 minutes of sonication (figure 3). Analysis of 2PM 5-minute timeseries data strongly suggests that capillaries are opened soon after FUS, but that larger vessels are not open after FUS (figure 3). Cross-sectional MRI study results show that the BBB remains open at 48 hours-post FUS in both WT and 5X-FAD mice (figure 4). T1-weighted imaging also shows that the BBB closes within 2-weeks (figure 4). No significant changes in volume of BBB opening or image intensity in the region of BBB opening were observed between WT and 5X-FAD mice. DCE analysis is on-going and will be compared to immunohistochemistry involving aquaporin, glial proteins and amyloid plaque burden in the same brains (figure 5).

Discussion and Conclusion

The main observations of this study are that the capillaries are preferentially opened by FUS and that there were no differences in BBB dynamics between wild type mice and the 5X-FAD mouse model of AD. Future directions and ongoing work include evaluation of kinetic analysis between strains and histologic evaluation of the aquaporin, glial cell proteins and amyloid plaque burden in the same brains. Taken together, the combination of 2PM and contrast MRI has advanced our understanding of FUS BBB opening and closing mechanisms and dynamics.

Acknowledgements

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References:

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Figures

Figure 1: 2-photon Microscopy Experimental Setup. The mice received a Rhodamine-B injection prior to imaging. FUS was applied between image acquisitions. FITC was injected during a fast timeseries.

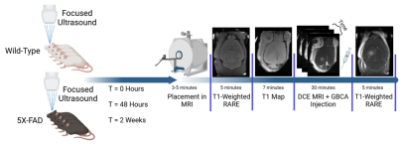

Figure 2: Experimental setup for cross-section study. Wild-type and 5X-FAD Alzheimer's Disease Mice were split into 3 groups each (6 groups total) and received FUS. After FUS, mice were imaged in the MRI at various timepoints after FUS. Mice were sacrificed and prepared for IHC after MRI.

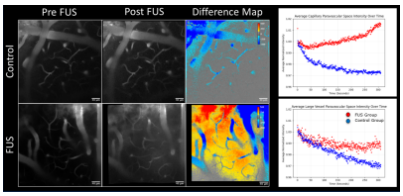

Figure 3: Pre and Post-FUS Z-stack projections in both FUS and Control groups. Difference maps indicate dye extravasation (red/orange) or signal decay over time (blue). Graphs depict the average intensity in capillary paravascular spaces and large vessel paravascular spaces.

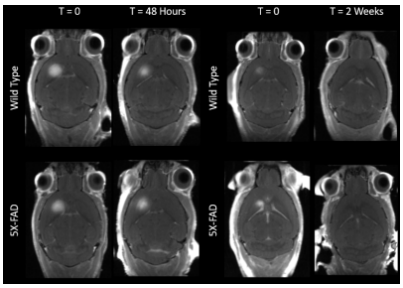

Figure 4: T1-weighted Gadolinium-based contrast agent enhanced images immediately after FUS, 48 hours after FUS, and 2 weeks after FUS. Wild-Type and 5X-FAD mice are depicted.

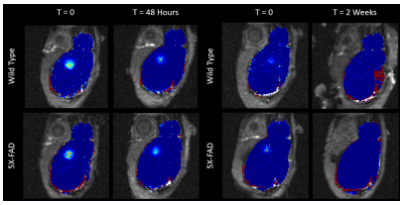

Figure 5: Dynamic contrast enhanced images immediately after FUS, 48 hours after FUS, and 2 weeks after FUS. The calculated Ktrans map is overlayed onto the raw DCE images. Wild-Type and 5X-FAD mice are depicted.

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