Magnetization Transfer Contrast MRI as a Diagnostic Tool for Alzheimer's Disease

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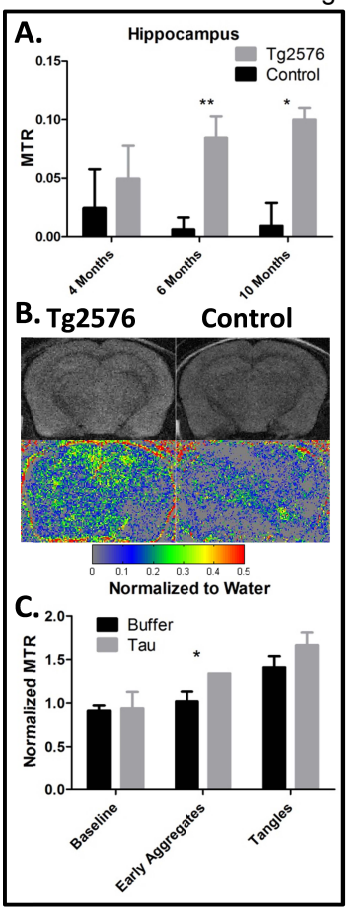
Introduction: Alzheimer's disease (AD), the most common form of dementia, is an incurable and terminal progressive neurodegenerative disease. The two predominant pathophysiological hallmarks of AD include amyloid plaques and neurofibrillary tangles. However, detecting either of them *in vivo* has proven difficult. Previous work has shown that neurological and behavioral deficits precede either of these hallmarks[1]. Therefore, imaging techniques that can detect afflicted subjects prior to plaque formation could be greatly beneficial for diagnosis of AD. Magnetization Transfer Contrast (MTC) is a Magnetic Resonance Imaging (MRI) technique to specifically detect changes in macromolecule concentration. In this work, we show that MTC MRI is sensitive to AD progression as seen in an AD mouse model: the Tg2576 mouse model. The Tg2576 mouse overexpresses a mutated form of amyloid precursor protein with a familial AD mutation and exhibits accumulation of amyloid as early as 4 months and eventual plaque formation as early as 10 months of age[2]. This mouse model does not present other hallmarks of AD like neurofibrillary tangles and neurodegenration[2]. Our results also show that the source of the MTC signal is not amyloid but rather likely to be tau aggregation.

Materials & Methods: *Mouse Imaging:* We examined Tg2576 animals and control littermates (N=4&5) at 4, 6, 10, and 12 months of age. Mice were anesthetized by isoflurane gas at a 5% in oxygen and placed into a mouse holder where they were kept under anesthesia at a nominal 2% isoflurane in oxygen. Imaging was performed utilizing a Bruker Avance Biospec, 9.4 T spectrometer; 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. During imaging the animal body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY). T2 weighted images were taken before MTC imaging to locate ideal MTC slice placement. An additional T2 weighted image of the MTC slice was taken to visualize the anatomy. MTC imaging pulse sequence comprised a pre-saturation square pulse at the designated offset frequency followed by a RARE sequence with TE/TR=8.14/1512 msec with Rare Factor=8. Images were recorded with a 256x256 matrix, Field of View=2x2 cm, slice thickness=1mm, and average=2. Pre-saturation off-resonance pulses ranged from 0 to 20 kHz for 12 month old mice. Only the 20 kHz offset was utilized for all other age points as it proved to be the only relevant offset. A reference image was also taken with the same parameters except the saturation pulse.

Protein Imaging: We tested the MTC properties of synthetic amyloid beta (1–42) (AnaSpec, San Jose, CA) and recombinant human Tau 2N4R (rPeptide, Bogart, GA). Amyloid beta was aggregated at a 0.1 mg/mL concentration at 37ºC. Tau was aggregated at a 0.3 mg/mL concentration in the presence of 10 µM heparin at 37ºC. For both, the same sample was imaged before aggregation and then at multiple time points afterwards to represent different stages of aggregation. As a control we imaged the buffer solution without any of the protein. *Data analysis:* Magnetization Transfer Ratios (MTR) in the form of *MTR = (Unsaturated - Saturated) / Unsaturated* were calculated. Pixel by pixel MTR calculations were performed in MATLAB (The Mathworks, Natick MA) to generate pseudo-colored images. Region based MTR calculations were also performed in Matlab for quantification. Graphs and statistical analyses were conducted on the region-based calculations with Prism (GraphPad Software, San Diego, CA).



Results and Discussion: We quantified the MTR results in the cortex and hippocampus of our mice. Figure 1A shows the results for the hippocampus at the 20kHz offset for ages 4 to 10 months. The same mice were imaged at each age point. The genotypes were found to be significantly different in each case by Repeated Measures ANOVA. Student's t test were performed for each individual age point. * is P<.05 and ** is P<.01. Figure 1B shows a representative anatomical T2 picture and MTR map for each genotype at 10 months. Pictures were aligned manually for ease of comparison. We saw no appreciable MTR signal in the amyloid beta samples (Data not shown). Figure 1C shows the MTR results for the Tau sample normalized to a water phantom to reduce variability.

Conclusion: The goal of this study was to test if MTC MRI would be sensitive to molecular pathology changes that are seen early in AD. Our results show that MTC MRI was able to detect differences in the Tg2576 mouse model well before plaque formation. Our initial hypothesis was that the signal was due to amyloid accumulation but we were unable to observe an MTC signal from aggregated amyloid. However, we were able to see an MTC signal from early aggregates of Tau that offers a mechanistic explanation for our results in the Tg2576 mouse model. This approach could potentially be used in the clinic as an early diagnostic test for AD and/or other Tauopathies.

References: 1. Smith, K.D.B. et al. *NeuroImage* **35**, 1401-1408 (2007). 2. Hsiao, K. et al. *Science* **274**, 99-102 (1996).