Magnetization Transfer Contrast (MTC) MRI for the Detection of Amyloid Accumulation in Alzheimer's Disease

C. J. Pérez-Torres[1,2], and R. G. Pautler[1,2]

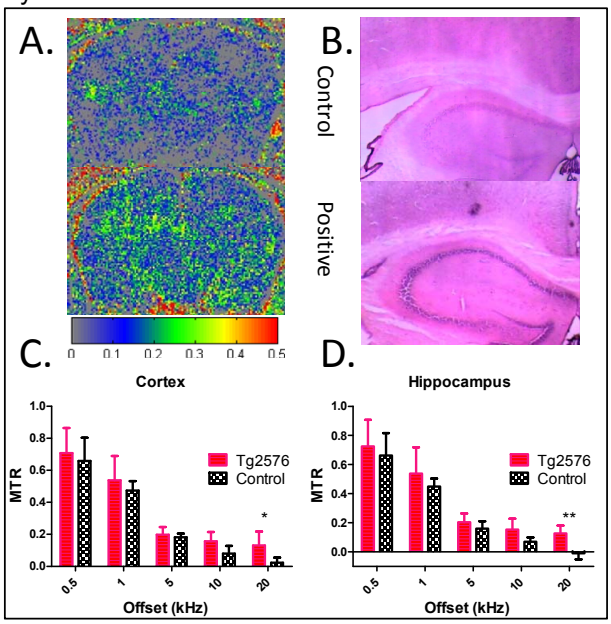
[1]Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, Texas, United States, [2]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas, United States

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 these hallmarks *in vivo* has proven difficult. Amyloid accumulation before plaque formation is also believed to be a causative factor in AD[1]. Therefore, imaging techniques that can detect amyloid accumulation prior to plaque formation could be greatly beneficial for diagnosis and monitoring 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 large changes in amyloid concentration 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].

Materials & Methods: *Imaging Methods:* Aged (12 months) Tg2576 animals (N=4) and control littermates (N=5) 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. A reference image was also taken with the same parameters except the saturation pulse.

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).

Immunohistochemistry: After all imaging experiments were finished the mice were perfused intracardially with 4% paraformaldehyde. The brains were then collected, cryoprotected in 30% sucrose and sections were cryocut at 30µm thickness. Sections were then stained with a floating section protocol with 6E10 antibody (Signet) in conjunction with M.O.M. kit (Vector Labs) which contained the secondary antibody. Finally, diaminobenzidine (Vector Labs) was used to visualize the immunostain. Section were counterstained with hematoxylin and eosin. Pictures were taken on a Motic dissection microscope at 60x magnification with a Moticam 480.



Results and Discussion: Figure 1A shows the MTR maps of the whole brain at the 20 kHz offset of a representative animal from each genotype. Figure 1B shows the representative 6E10 staining of the hippocampus at around the same area as the MRI image. The increase in 6E10 staining for the Tg2576 animal seen in Figure 1B correlates with the increased MTC signal in Figure 1A. Regions of interest were drawn of the hippocampus, cortex and whole brain. Each region was analyzed separately. Figure 1C-D shows the quantification results for the cortex and hippocampus respectively. We found a significant difference between the genotypes by ANOVA for cortex (P<.05) and hippocampus (P<.01) and specifically we found a significant difference between the genotypes by One Sided Student's t test at the 20 kHz offset for cortex (P<.05) and hippocampus (p<.01). We found no significant difference when we looked at the data for the whole brain.

Conclusion: We can observe differences in the MTC signal that could potentially be linked to accumulation of amyloid in a model of advanced Alzheimer's disease (AD). We are currently testing if this hold true before plaques develop. This methodology has potential as a way to track disease progression in AD. More importantly, this approach could potentially be used in the clinic as an early diagnostic test for AD.

References: 1. Shankar, G.M. et al. *Nat Med* **14**, 837-842 (2008). 2. Hsiao, K. et al. *Science* **274**, 99-102 (1996).