The Role of Iron in T_2* Contrast and Transverse Relaxation of Beta-Amyloid Plaques in Alzheimer's Disease

M. D. Meadowcroft[1,2], J. R. Connor[3], and Q. X. Yang[1,3]

[1]Radiology - Center for NMR Research, Pennsylvania State University - College of Medicine, Hershey, PA, United States, [2]Neural and Behavioral Sciences, Pennsylvania State University - College of Medicine, Hershey, PA, United States, [3]Neurosurgery, Pennsylvania State University - College of Medicine, Hershey, PA, United States

Introduction: Understanding the transverse relaxation and image contrast associated beta-amyloid (Aβ) plaques in Alzheimer's disease (AD) has been of great interest for NMR research. It has conventionally been believed that MR contrast associated with Aβ is due in large part to focal iron associated with plaque deposition. Our previous research (1, 2) has helped to illustrate that both iron and plaque morphology are synergistic in their decreasing of transverse relaxation rates in Alzheimer's and the Aβ generating APP/PS1 transgenic model plaques. Alzheimer's plaques with high iron content are observed on MR images while transgenic animal plaques with a minute amount of iron associated with them were still discernable on MR images. The goal of this research is to further understand the intricacies of how plaque morphology and associated iron content is related to T_2 and T_2* transverse relaxation of human AD plaques. Detailed histological MR examination in comparison to traditional histology methods of human AD tissue samples that have been treated with an iron chelator to reduce iron content is described.

Methods: Entorhinal cortex brain tissue samples from clinically and histologically determined AD subjects (N=5) were used throughout the study. Tissue samples fixed with paraformaldehyde and cryogenically protected with sucrose were cut at 60μm using a cryostat and placed in phosphate buffered saline (PBS) to eliminate any residual fixative and sucrose. Two tissue slices cut sequentially from the same sample were used for each MR and histological experiment. Slices were placed on standard slides, circled with a hydrophobic barrier pen and placed in a high humidity chamber. For iron(III) chelation, one tissue sample was treated with a 0.5ml 76000 μM Deferoxamine Mesylate (DFO) (#D9533, Sigma Aldrich, St. Louse, MO) solution in dH_2O for 24 hours while the other sample was treated with 0.5 ml of dH_2O under similar conditions. Samples were then rinsed in PBS and placed adjacent to one another for micro-imaging within the 7.0 T histological coil (1, 2). For MR imaging an eight echo T_2* MGE sequence, eight echo T_2 MSME and a seven echo variable timing T_1 scans were utilized with a matrix of 512^2 and a final pixel resolution of 45μm x 45μm in the through-plane direction. Following histological MR imaging, the slices were co-stained with Thioflavin-S for Aβ plaque detection and a Perl's – Diaminobenzene stain for ferric iron. MR images were compared to histological stains while R_2* and R_2 parameter maps were created and detailed relaxation measurements of individual plaques and regions of interest were analyzed.

Results: Figure 1 shows a T_2* weighted MR image (A, B) and low magnification (C, D) images of AD tissue samples untreated (A, C) and treated (C, D) with the iron chelator DFO. The untreated tissue shows characteristic MR hypo-intensities associated with Aβ plaques and iron deposition. The DFO treated tissue exhibits less iron in the cortical gray matter and white matter tracks as seen in both the MR and microscope images. Figure 2 shows the same tissue samples histologically MR scanned (A, D), stained for iron (B, E) and plaque deposition (C, F). Tissue samples not treated with DFO show hypo-intensities on the MR images (A, and enlargements) that are associated with both high iron content (B) and Thioflavin-S positive plaques (C). Samples treated with DFO show similar hypo-intensities in the gray matter (D, and 40x enlargements) that are associated with Aβ plaque deposition (F) but are negative for iron staining (E).

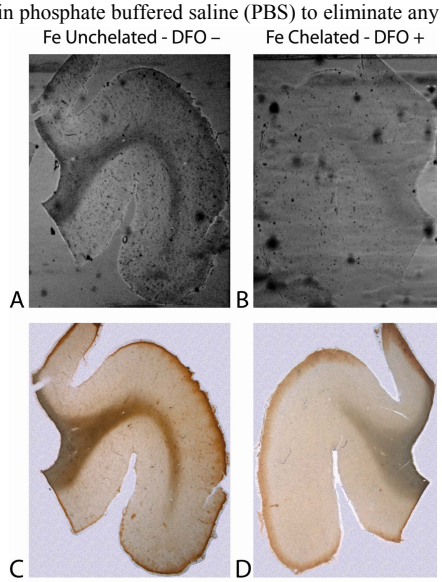

Figure 1: T_2* weighted MR images (A, B) and low magnification microscopy (C, D) images of the same human AD tissue samples untreated (A, C) and treated (B, D) with the iron chelator DFO. The MR and microscope images of non-chelated tissue shows characteristic MR hypo-intensities and iron staining, especially in the gray matter cortical region. The DFO treated tissue shows less iron throughout but and less contrast associated with iron in white matter tracks but still has MR hypo-intensities in cortical regions.

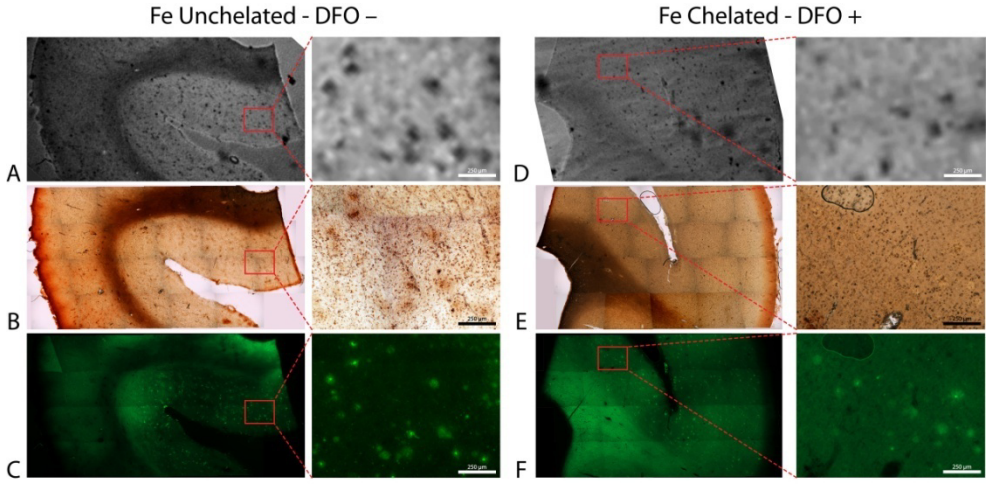

Figure 2: T_2* weighted MR images (A, D) and microscopy images for iron (B, E) and fluorescent Thioflavin-S positive Aβ plaques (C, F) of the same human AD tissue samples untreated (A, B, and C) and treated (D, E, and F) with the iron chelator DFO. The enlarged 40x regions of the DFO untreated tissue shows hypo-intensities in the MR images that are associated with high iron content and Aβ plaques. The DFO treated tissue shows a lack of positive iron staining associated with Aβ plaques but still exhibits hypo-intensities on the MR images associated with plaque deposition. The scale bars in the enlarged images are 250 μm in length.

Discussion: Our previous research illustrated that transverse T_2* MR contrast seen in images of Aβ plaques is associated with both plaque morphology and iron content, due to the inherent lack of iron associated with the transgenic APP/PS1 model Aβ plaques. It was not clear as to how this pertained to the human AD tissue samples as they all contained focal iron regions associated with plaque deposition. In utilizing the current chelation approach we have diminished the iron associated with the Aβ plaques while still showing that plaques are discernable on the transverse MR image sets, as seen in the histology and MR images. Similar to the previous APP/PS1 data, Aβ plaques in human tissue fall under our proposed dual relaxation mechanism caused by both iron and plaque morphology. The data indicate that iron content, while a factor in the MR imaging of plaques, alone is not responsible for the hypo-intensities seen on the MR images and that the dense fibrillar nature of the Aβ plaques has a major role in shortening of the transverse relaxation in human AD tissue. The iron content and plaque composition synergistically cause the additive effect upon transverse relaxation. R_2* magnitude images and T_1 parametric maps indicate alterations in T_1 relaxation in human Aβ plaques as well (not shown). This data represents a required step forward in understanding the relaxation mechanisms associated with Aβ plaques for future utilization in clinical and translational research.

References: 1 – Meadowcroft *et al*. Mag. Reson. Med. 2007; 57(5): 835-841, 2 – Meadowcroft *et al*. JMRI 2009 May; 29(5): 997-1007.