Micro MR Imaging of Beta-Amyloid Plaques and Co-Registration with Iron Deposition and Histological Analysis in both Human Alzheimer's Disease and APP/PS1 Transgenic Mice

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Introduction: Our prior study involving the development of a histological coil addressed a long-standing difficulty in co-registration of MRI data with histological tissue images (1). Using this coil, a one-to-one relationship between MRI parametric maps and histological chemical stains can be achieved. Aberrant regulation of iron leading to its focal concentration in brain tissue is believed to be associated with the neurodegenerative processes found in Alzheimer's disease (AD) and co-localization of iron with the beta-amyloid (Aβ) plaques is accompanied by endoplasmic reticulum stress induced apoptosis, DNA oxidation and cellular damage in cells adjacent to plaques (2). A longstanding problem with magnetic resonance imaging (MRI) of beta-amyloid plaques has been the understanding of how transverse T_2 and T_2* relaxation is effected by the amyloid protein and matrix surrounding it. Using the histological coil, here we present data demonstrating unambiguously that MR images and parametric maps closely correlate with both the hallmark Beta-Amyloid (Aβ) plaques and iron content in human AD, though only with the Aβ plaques in transgenic APP/PS1 mouse tissue found by histological staining. The mechanism of how Aβ plaques are viewable with and without focal iron regions utilizing MRI is studied.

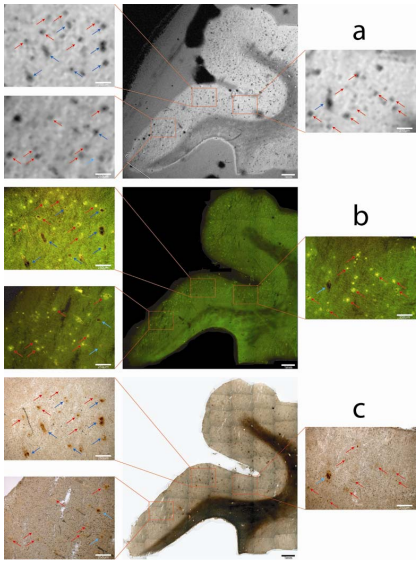

Figure 2: (a) T_2* weighted image, (b) thioflavin-S and (c) Perl's iron stain of the same tissue section from the entorhinal cortex of an Alzheimer's subject. The MR image and magnifications show focal hypo-intense regions that co-register well to both plaque location in (b) and focal iron location in (c) (red arrows). Blue arrows are regions seen in the MR image associated with focal iron but not with plaques.

Methods: Human brain tissue samples were dissected from the entorhinal cortex in histological and clinically determined AD subjects (N=5) and age-matched controls (N=4). The tissue was fully fixed in 4% paraformaldehyde and then placed in sequential graded 10%, 20%, and 30% sucrose for cryogenic protection. Tissue sections were cut with a Leica cryostat at a thickness of 60 µm and trimmed to approximately 1cm x 1cm. Transgenic mice (N=5) inserted with human APP/PS1 and age matched control cage mate C57BL/6 mice (n=3) were allowed to age 24 months till Aβ plaque formation. Animals were euthanized and then were transcardially perfused with cold Lactated Ringer's solution followed by 4% paraformaldehyde. Whole brain tissue was harvested and placed overnight in 4% paraformaldehyde to allow for full tissue fixation and then placed in sequential graded 10%, 20%, and 30% sucrose for cryogenic protection. 60 µm tissue sections were cut at approximately Bregma = -2.80mm. Histological MR imaging was accomplished on a 7.0T Bruker BioSpec magnet and the histological coil (1). 60µm tissue samples were floated in PBS to leech any residual formalin and sucrose out of the tissue, were encased between two #1 glass coverslips in PBS and inserted into the histological coil (for full procedure see (1)). Multi spin-echo (MSME) for T_2 mapping, and a rapid gradient echo multi-echo (MGE) protocol for T_2* mapping were acquired with in-plane resolution of 45 x 45 µm and a through plane of 60µm. After MR imaging, the tissue samples were removed from the coverslips and co-stained with Thioflavin-S for Aβ detection and a modified Perl's with 3'3-diaminobenzine enhancement for iron content. High resolution images of the stained tissue were taken under visual light spectra and fluorescence at 430nm excitation and 550nm emission for respective iron and Aβ plaque analysis. To identify calcium in the tissue sections of both species a modified 2% Alizarin red S and Thioflavin-S stain was employed.

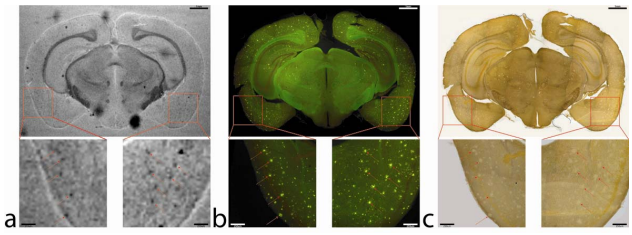

Figure 1: (a) T_2* weighted image, (b) thioflavin-S and (c) Perl's iron stain of the same tissue section from an APP/PS1 animal. The MR image and magnifications below show focal hypo-intense regions that correspond to plaque locations in (b) but not with iron location in (c).

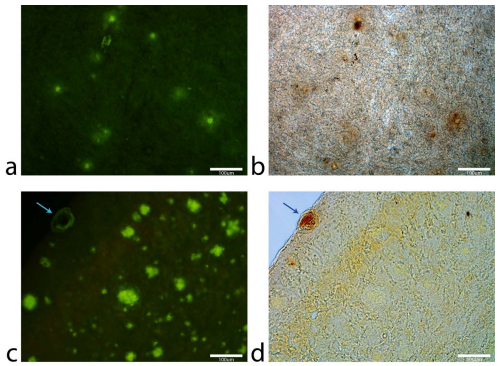

Figure 3: 100x magnification Human AD (a) thioflavin-S and Perl's stain (b) and APP/PS1 (c) thioflavin-S and Perl's stain (d). The relationship between plaques and iron is clearly seen with the AD samples while no iron is seen associated with the APP/PS1 animals. The arrow in (c) and (d) is an iron stained capillary illustrating the positive stain for iron. APP/PS1 plaques show a larger and denser amyloid core.

Results: Figure 1 shows the T_2* weighted image (a), Aβ plaque (b) and focal iron staining (c) in an APP/PS1 mouse tissue section. The MR image illustrates hypo-intensities in the periform cortex of the APP/PS1 animal which co-register well to the large Aβ plaques with a minimal diameter of 40µm. The iron stain indicated very little ferric iron present in the location of the plaques, yet stained positive for known high iron regions. T_2* weighted image (a), Aβ plaque (b) and focal iron staining (c) from human Alzheimer's tissue within the entorhinal cortex is shown in Figure 2. The hypo-intensities in the MR image and magnifications co-register well with plaque location and focal iron. Plaques that are 38µm in diameter and have iron associated are seen in the MR image while larger plaques without iron are not seen. Figure 3 shows a 100x magnification of human AD tissue (a,b) and APP/PS1 tissue (c,d). The plaque and iron stains in the AD tissue illustrate a close relationship between plaque and iron location. In opposition are the APP/PS1 tissue samples which show a void of iron where plaques are located. APP/PS1 plaque morphology differs from the AD plaques in that they have larger and denser amyloid cores. Calcium staining for both species indicated a positive relationship between calcium and plaque location.

Conclusions: Individual plaques can be resolved in MR images with the histological coil in both human AD and APP/PS1 mouse tissue samples. Histological stains indicate that the presence of focal iron is the dominate feature in observing human AD plaques. Conversely, large plaques that have no associated iron load within the APP/PS1 animals are viewable with MR images. The relationship between plaque morphology, size of the plaques, their co-registration with iron, and the potential hydrophobic nature of the backbone Aβ peptides within plaques all seem to play an important role in how visible the plaques are with MR imaging techniques.

References:

1 – Meadowcroft *et al.* Mag. Reson. Med. 2007; 57(5): 835 – 841

2 – Rottkamp *et al.* Free Radic. Biol. Med. 2001; 30(4): 447 – 450