MRI Assessment of Aß-iron complex in PS/APP mouse Brain

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Introduction: Reduced mean T_2 values in the hippocampus and cortex of 18 month old PS/APP mice in vivo [1] and detection of Aβ plaques in vivo and in vitro without the use of any contrast reagents [2;3] have been reported. The PS/APP transgenic mouse is a model of Alzheimer pathology overexpressing both mutant human APP and PS1, and showing an early and extensive amyloid deposition [4]. The presence of iron in Aß plaques, in this model, has been suggested to be a significant factor responsible for reducing T_2 and the source of the intrinsic MRI contrast of Aß plaques seen in these animals. Recently, it has been demonstrated that iron co-localizes with Aß plaques in the brains of this mouse model of AD pathology [2;5]. However, the form of iron cannot be specified by Perls' stain alone. Therefore, in this study, we investigate the relationship between MR hypo-intensity signal and Aß-iron complex determined by Perl's stain and anti-ferritin immunohistochemistry in the PS/APP mouse brains. In addition, we implemented a new MRI-based technique referred to as the Magnetic Field Correlation (MFC) Imaging [6;7], which is known to be very sensitive to the presence of iron.

Methods: A total of seven 20 month-old mice (two non transgenic controls and five PSAPP transgenic) were perfused with phosphate-buffered saline (PBS) (pH 7.4) through the left cardiac ventricle, followed by 10% buffered formalin. After perfusion fixation, the brains were removed, embedded in agar (0.4%), positioned at the center of a plastic tube and tightly sealed for MR measurements. All experiments were performed on a 7 T MR system (SMIS, Guilford, UK). High-resolution MR images were acquired using a multi-slice fast spin-echo (FSE) sequence with navigator correction for Nyquist-ghost reduction. The data acquisition parameters are: base echo time (TE) = 10 ms, repetition time (TR) = 3500ms, flip angle =90°, FOV =12.8mm ×12.8mm, matrix: 200x196, number of slices = 25, effective echo time = 10 ms, and echo train length = 4. MFC images were acquired using an asymmetric dual spin-echo pulse sequence. Imaging parameters were TE/TR = 30/3000 ms, FOV=12.8mm, slice thickness 200 µm, matrix=64 x 64, and echo time shifts of 0, ±3, ±6, ±7.8 ms. MFC maps values were calculated by non-linear least square fit of signal intensities on echo time shifts, based on the relationship between MFC and asymmetric echo shift time shift [6]. Regions of interest (ROIs) were manually drawn in the cortex (Ctx), hippocampus (Hippo), thalamus (Thal) and globus pallidus (Gpa). To estimate MFC values, the signal intensity values were computed prior to the fitting process. After imaging the brains were submitted to immunohistochemical assessment of iron (Perl's Stain and ant-ferritin (Sigma) and Aß (Campbell-Switzer) at the (NeuroScience Associates, Knoxville, TN) following a standard protocol [5;8] that included the brains been treated with 20% glycerol and 2% dimethylsulfoxide to prevent freeze-artifacts and subsequently embedded in a gelatin matrix using MultiBrain Technology and sectioned coronally at 40µ.

Results and Discussion: Shown in Figure 1 is MR microscopy (A), Perl's Stain (B) and anti-ferritin (C) images. The MR microscopy images showed numerous hypo-intensities, corresponding to Aß plaques, visible throughout the PS/APP mouse brain, including the cortex and hippocampus. As previously reported [5], Perls' stain labeled the majority of Aß plaques. In the same way, the anti-ferritin immunochemistry labeled primarily amyloid plaques. Therefore, confirming that ferritin-iron contained in the Aß plaques is the main source of MRI contrast and the significant factor responsible for the reported reduction of T_2. Our data also show that the MFC values are somewhat higher in the cortex, globus pallidus, thalamus and hippocampus of PSAPP mice compared with non-transgenic controls (Table 1). Although the number of mice is not enough for a meaningful statistical analysis (data are part of an ongoing project), the data clearly show that MFC correlates with the increase of iron content that occurs in this AD pathology mouse model. Our data confirm that the source of MRI signal changes, including the MFC changes, is related to the presence of Aß-iron deposition. These results suggest that MFC imaging can be a potentially useful tool for assessing brain iron and the role of brain iron disruption in the pathogenesis of Alzheimer's Disease. Further studies are being performed to correlate MRI quantification indices, including MFC and the degree of Aß-iron complex measured by biochemical (total iron concentration) and immunohistochemical (Aß load, iron density index and iron fractional volume index) methods.

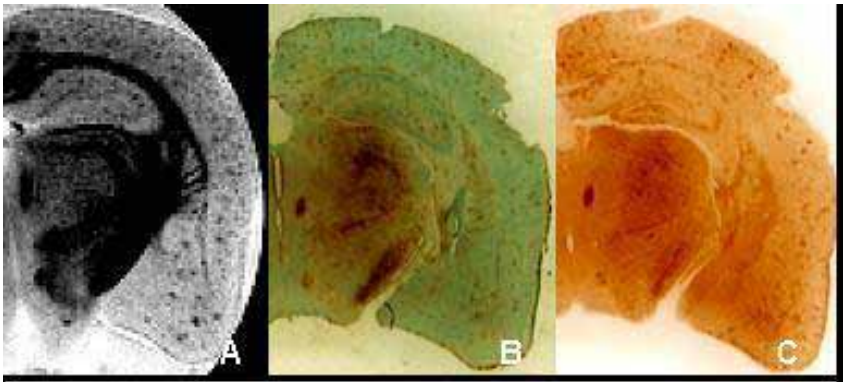

Figure 1: MR microscopy (A), Perl's Stain (B) and ant-ferritin (C) images. The MR microscopy images showed numerous hypointensities, corresponding to Aß plaques, visible throughout the PS/APP mouse brain, including the cortex and hippocampus. Perls' stain and anti-ferritin immunochemistry labeled the majority of Aß plaques.

Table 1: Regional MFC values (s^{-1}) in Ntg and PSAPP mice Brain

		Ctx	Gpal	Thal	HippoT
Ctr (2)	mean	74.42	507.46	180.97	67.10
	sdv	19.41	68.10	26.21	12.85
PSAPP (5)	mean	90.48	552.84	285.52	101.05
	sdv	22.02	167.78	195.62	23.19

References: **1**. Helpern JA et al. Magn Reson Med. 2004; 51, 794-798. **2**. Lee SP et al. Magn Reson Med. 2004; 52, 538-544. **3**. Jack CR et al. Magn Reson Med. 2004; 23;52(6):1263-1271. **4**.Holcomb L, et al. Nat Med 1998; 4:97–100. **5**. Falangola MF at al. Neurochemical Research 2005; 30: 201-205. **6**. Jensen JH and Chandra R. Proc. Intl. Soc. Magn. Reson. Med. 2002;10:2297. **7**. Lee SP, et al., Proc. Intl. Soc. Mag. Reson. Med. 2005;13 :421 8. Campbell, S.K et al. Society for Neuroscience Abstracts, 1987; 13: 189.9.

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