MR Imaging of Cu2+ Treated Alzheimer disease tissue

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Introduction

Alzheimer's disease (AD) is characterized by amyloid deposits within the neocortical parenchyma and the cerebrovasculature. Amyloid-beta (Abeta) is cleaved out of a ubiquitously expressed parent protein, the amyloid protein precursor (APP). APP possesses copper/zinc binding sites in its amino-terminal domain and in the Abeta domain (affinity Zn2+>Cu2+>Fe3+). Studies have implicated copper, iron, and zinc as key mediating factors in the AD pathophysiology (1, 2).

The remaining binding-sites in amyloid deposits may be utilized for MR imaging of AD tissue. We have tested the feasibility of using Cu2+ to assess amyloid deposits via those sites. Our preliminary results include MR imaging of Cu2+ treated AD tissue at 3T and histologic validation.

Methods

Human tissue sample: Four formalin fixed human forebrain tissues, two normal and two AD changes stage 6, were obtained from Harvard Brain Tissue Resource Center. A normal and an AD tissue samples were immersed with 50 times volume of saline containing 0.1 mM $CuSO_4$ (physiological level) under 4C for eight days. The Cu2+ solution was changed every day up to day eight. The control group (a normal and an AD) was immersed in 50 times volume of normal saline, and changed the saline similar to the Cu2+ treated group. The treated tissues were stored in normal saline for MR imaging.

MRI: A clinical 3T whole body scanner (Trio, Siemens AG, Erlanger, Germany) using an extremity volume coil for transmit and receive. Tissue samples were placed in a rectangular jar container. T1weighted and T2w images were acquired with a turbo-spin echo (TSE) sequence. T1w images were acquired with parameters TR/TE 500/9.2, slice thickness 1 mm, matrix 256x256. T2w images were acquired with parameters TR/TE 6000/97, slice thickness 1mm, matrix 256x256. T1 of the tissue samples was measured using inversion-recovery scheme with turbo-spin echo readout (TI 25, 50, 75, 100, 200, 300, 500, 750, 1000 ms and TE/TR 12/8000) and T2 was measured using a TSE sequence (TE 12, 25, 37, 50, 62, 87ms, TR 4500ms). Region-of-interests (ROIs) were placed in gray matter and white matter to compare the difference between AD and normal.

Histologic validation: After MR scans, the two Cu2+ treated human tissue samples were sliced as 80µm sections and affixed to glass slides for histologic examination. Amyloid deposition in plaques and cerebrolvasculature was characterized by thioflavin-S stain. Tissue-bound copper was emerged by modified Mallory's method, and was evaluated by optical density. Optical images were acquired with AxioVision 3.1 (ZEISS) and analyzed with ImageJ. ROIs were placed in the optical images of gray matter to estimate Cu2+ uptake difference in normal and AD tissue.

Results

AD and normal human tissue samples showed similar profiles, higher signal intensities in cortical gray matter than in white matter with relatively blurred intermediate regions, in both T1w, and T2w images (Fig. 1). AD cortical gray matter clearly showed atrophy compared to normal. Cu2+ treated samples show a much clear separation between gray and white matter and between disease and normal. T1w signal intensity of treated AD gray matter is ~21% lower than that of normal (Fig. 1a-d) while signal intensity of untreated AD gray matter is only ~6% lower. No significant difference in signal intensities were observed between the untreated AD and normal samples. Biexponential T1 decays were observed in the tissue samples and both T1 and T2 values are summarized in table 1.

Histologic images confirmed Cu2+ accumulation in gray matter of the tissue samples (Fig. 2a-b). AD gray matter appears to have more Cu2+ accumulated spots among the samples under investigation. Comparing to adjacent slices with thioflavin-S stain, those copper positive spots include plaques or cerebrovasculature (fig. 2b to c). We have also observed Cu2+ attached residual red blood cells (RBCs) within some vessels. Both plagues and cerebrovascular amyloid angiopathy have extracellular deposition of Abeta and involve in the pathological changes of Alzheimer's disease (3). Morphologically, Cu2+ displayed strong affinity with the plaque core, and showed inferior binding ability with the rim area (fig. 2d-e). Averaged signal intensity of ROIs placed on the AD gray matter is approximately 18 % lower than that of normal in the Cu2+ treated samples, which is consistent with T1w images.

Discussion

The results of this study confirmed gray matter atrophy in AD brains (3) and Cu2+ uptake in amyloid deposits (1, 2). T1 and T2 shortenings in Cu2+ treated tissue samples are likely due to uptake of Cu2+. This uptake appears to be concentrated in gray matter probably via available binding sites on amyloid deposits and blood vessels as well as some residual RBCs and may be utilized in animal studies of AD.

References

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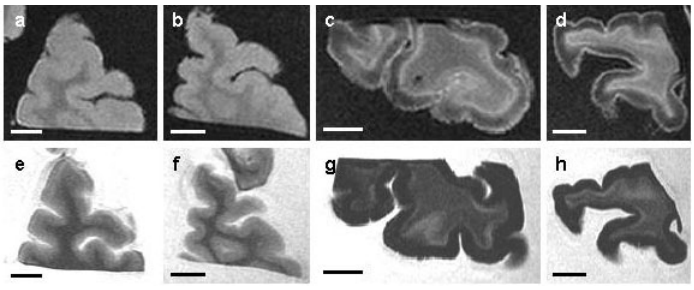

Figure 1: Typical T1w (a-d), and T2w (e-f) image of Cu2+ treated (c-d,g-h) and untreated (a-b,e-f) AD (b,d,f,h) and normal (a,c,e,g) tissue. scale bar=10mm.

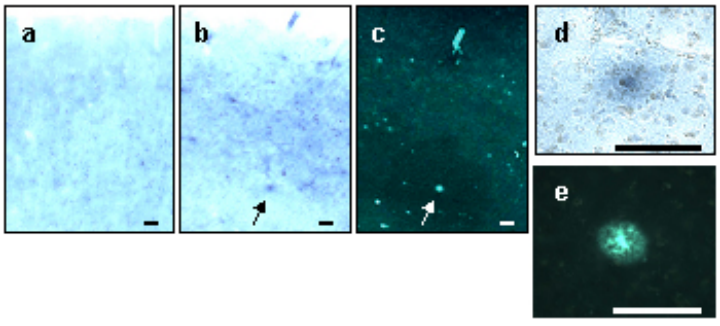

Figure 2: Histologic validation of Cu2+ targeting on AD related amyloid deposits a=normal, b=AD, c=thioflavin-S of AD, d,e=expanded region, scale bar=100µm, arrow= $copper^{+}$/thioflavin-S^{+} labeled plague.

Table 1 T1 and T2 of gray and white matters in normal and AD brain tissue. For biexponential T1 decays, data presented as T1(short)/T1(long).

	normal		AD		normal+Cu		AD+Cu	
	Gray matter	White matter	Gray matter	White matter	Gray matter	White matter	Gray matter	White matter
T1 (ms)	332/1285	270/2315	418/1253	308/2268	85/1111	256/625	79/1111	270/625
T2 (ms)	88	52	86	56	23	51	22	57