Iron is a Prerequisite for Direct Visualization of Alzheimer's Plaques in Animal Models

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Introduction: Hypercholesterolemia is thought to play a role in triggering Alzheimer's disease. Rabbits fed diets rich in cholesterol exhibit neuronal accumulation of beta-amyloid (Aβ) and senile plaques (SPs) in their brains[1]. We recently showed[2] that New Zealand White rabbits fed 0.125-0.25% cholesterol-enriched diets for 27 months formed a specific Aβ plaque type with dramatic and consistent iron deposits. Furthermore, we demonstrated that these plaques can be directly visualized as signal voids using clinical-field strength (3T) MRI of the excised brains (Figure 1). Our goal in this study was to determine whether iron co-localization in Aβ plaques is a requirement for their visualization on 3T MRI. To accomplish this goal, we studied two additional AD models known to produce Aβ-rich plaques.

Methods: Rabbits fed a high-cholesterol (2% w/w) diet for a short time (10 weeks) (n=5)[1,3] and an AD transgenic mouse (TgCRND-8)[4] were dissected after transcardial perfusion rinse, fixed in 4% paraformaldehyde for 24 hours, and then mounted in containers suitable for high resolution ex vivo MRI. We performed MR imaging at 3T using a protocol that had been previously optimized for senile plaque detection in long-term, low-cholesterol-enriched rabbit brain imaging (3D FIESTA pulse sequence; 96 minutes; 66x66x100 μm^3; TR/TE, 20/10 ms; FA, 20°; BW, 18 kHz; phase cycling number/recon, 10/sum-of-squares)[2]. Following imaging, 40 µm sagittal sections were cut with a vibratome and free floating sections were stained for both amyloid beta (Aβ-42 immunostaining) and iron (Prussian Blue).

Results; Unlike MR images of the long-term low-cholesterol-enriched rabbit model, MR images throughout the brains of the short-term 2% cholesterol-diet-enriched rabbits showed no obvious signal voids. Aβ-42 immunohistochemistry and Prussian Blue double staining revealed that SPs identified in these brains were relatively small and not associated with iron accumulation (Figure 2). Similarly, matched MR imaging of the AD transgenic mouse brain revealed no signal voids, and Aβ-42 and Prussian Blue stained sections showed abundance of Aβ-rich plaques with little associated iron. An additional finding was that the iron-poor plaques in this mouse model were significantly larger (20-140 µm, Figure 3) than those in the two different rabbit models (7-15 µm) (Figures 1 and 2). The rabbit plaques more closely resemble the "burned out" plaque type in human brain, which are not often seen in AD mouse models[5,6].

Conclusion: These results provide the first evidence that the long-term low-cholesterol-enriched rabbit model produces human-like senile plaques, which also co-localize with iron more consistently than two other established models of AD. In addition, we have demonstrated that 3T MRI is capable of detecting the SPs in the long-term low-cholesterol-enriched model of AD, but not in the other two models.

References:

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Figure 1

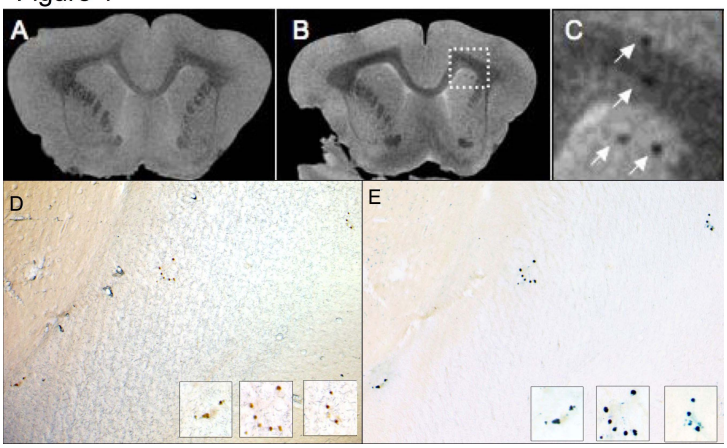

Figure 2

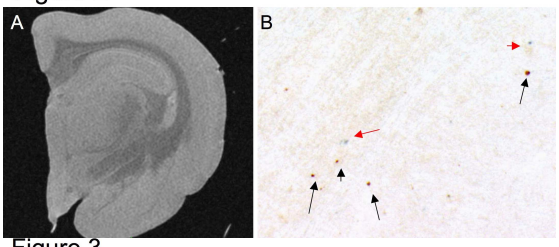

Figure 3

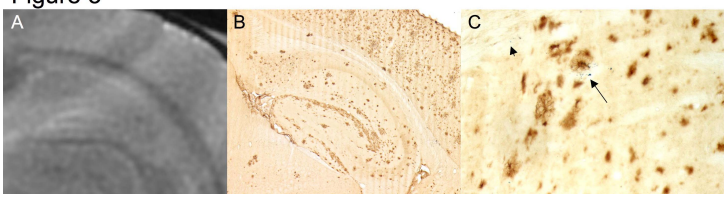

Figure 1. Brain MRI reveals the signal voids in the rabbits fed 0.125-0.25% cholesterol-enriched diets for 27 months (B,C) comparing with controls (A). Aβ-42 immunostaining (D), and Aβ-42/Prussian Blue dual staining (E) show the amyloid plaques with dramatic iron deposits. **Figure 2.** No obvious signal voids in MRI of the short-term diet rabbit brain (A). Aβ-42/Prussian Blue double staining (B) reveals that small size of SPs (black arrows) in the brains are not associated with iron accumulation (red arrows). **Figure 3.** MR imaging of the TgCRND-8 mouse brain reveals no signal voids (A). Aβ-42 (B), and Prussian Blue dual staining (C) in the brain exhibit the various sizes of dense-cored plaques and neuritic pathology, but no significant iron present. (Black arrow indicates the positive iron stains)