Monitoring Treatment Effects in Transgenic Mouse Model of Alzheimer's Disease using MRMI

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Introduction

Despite the enormous public heath impact of Alzheimer's Disease (AD), no disease modifying treatment has yet been proven to be efficacious and safe in humans. Several independent laboratories have reported that peripheral administration of anti-Aβ antibodies to AD transgenic mice, so-called passive immunization, significantly reduces amyloid plaque burden and improves memory performance.

In this work, the feasibility of using Magnetic Resonance Micro-Imaging (MRMI) to detect modification of plaque deposition (treatment effect) by passive immunization therapy is presented.

Methods

Six double transgenic (APP-PS1) mice 12 months of age at baseline were entered into a 2 month treatment study. Half (n = 3) were randomly assigned to treatment and half to placebo. Treatment consisted of weekly intra peritoneal (IP) injections of 10 mg/kg 2H6 anti-Aβ monoclonal antibody supplied by Rinat Neuroscience Corp.[1,2]. Placebo consisted of weekly IP PBS injections. All mice underwent MRMI studies using the T_2-weighted MRMI technique[3] at baseline, 1 month, and again following 2 months of treatment. At 2 months, the animals were sacrificed. *Ex vivo* MRMI, Thio-S histological staining, and quantification of plaque burden were performed. Plaque quantification was performed by a technologist who was blinded to treatment arm and timing of acquisition of the MRMI images. In order to cross validate the treatment results with a traditional automated plaque counting method for histologically stained tissue, Thio-S-stained sections (30 μm) were analyzed by a semi-automated image analysis technique (Figure). Ten sections were analyzed using image analysis software (Ziess Axiovision) throughout the retrosplenial cortex and the dorsal hippocampus of the right hemisphere. Amyloid burden was expressed as a percent of the total area occupied by plaques.

Results and Discussion

All mice survived the treatment study and all of the imaging examinations with no apparent difficulty. The results of the Ziess plaque image analysis software analysis, illustrated in Figure, are the mean (± SEM) of 10 sections per mouse for each brain region for three mice by treatment arm. A 3-way analysis of variance (ANOVA, SigmaStat; treatment x mouse x region) was performed followed by Student Newman-Keuls post-hoc multiple comparisons. A modest but statistically significant treatment effect (i.e., lower amyloid burden) was observed in the mice treated with antibody across both brain regions ($F(1,108) = 18.10$; $p < 0.001$). Student Newman-Keuls comparisons indicated that the amyloid burden was significantly lower in both brain regions in antibody-treated mice (cortex: $p < 0.02$; hippocampus: $p < 0.001$).

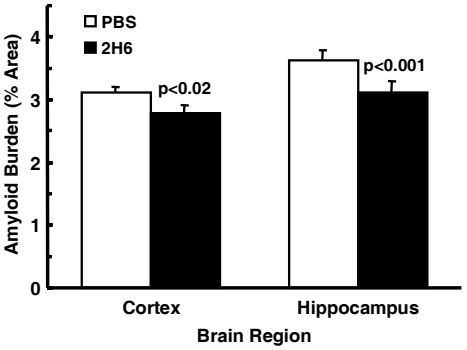

Figure. Automated plaque burden per unit area by semi-automated quantification: treated versus placebo mice.

Table illustrates mean (SD) plaque counts per mouse averaged across all 20 cortical ROIs for *in vivo* MRMI at baseline, month 1, and month 2, *ex vivo* MRMI at month 2, and Thio-S at month 2. A clear trend indicating plaque clearance/reduction in treated vs. placebo animals is seen in the Thio-S data which we take as the gold standard. Note also that results of the ROI based manual plaque counting method in Table match those of the automated Ziess plaque counting method in Figure, which serves as another source of external validation for the manual ROI based quantitative method for MRMI plaque load. *In vivo* baseline MRMI reveals a greater plaque burden in the treated than the placebo cohort at baseline. For this reason we report the data from treated animals in two forms: unscaled are the unadjusted average plaque counts per animal, while scaled values are adjusted by a factor of 0.93 – the ratio of plaque counts at baseline in the PBS cohort divided by the antibody treated cohort. The *in vivo* MRMI at both month 1 and 2, as well as the *ex vivo* MRMI reveal a trend toward lower plaque counts in treated vs. placebo mice.

Table. Plaque Counts in APP-PS1 Mice Treated with 2H6 Anti-Aβ Antibody (n = 3) or Injected with PBS Placebo (n = 3).

	In Vivo MRMI at Baseline	*In Vivo* MRMI at Month 1	*In Vivo* MRMI at Month 2	*Ex Vivo* MRMI at Month 2	Thio-S at Month 2
Treated (unscaled)	4.2 (1.3)	3.8 (0.5)	4.1 (1.0)	4.3 (0.4)	11.5 (2.6)
PBS	3.9 (1.0)	4.3 (0.2)	4.1 (0.6)	4.5 (0.6)	12.4 (0.4)
Treated (scaled)	3.9	3.6	3.8	4.0	10.7

These results indicate that a treatment effect was demonstrated via Thio-S stains, a gold standard method of plaque quantification. A trend in the direction of a treatment effect was seen with *in vivo* MRMI. Interestingly, the *in vivo* MRMI data indicate a drop in plaque count below baseline levels in treated mice at one month with a slight increase from month 1 to 2 which is what might be expected given that the natural history in these animals is a continual increase in plaque load with age. The *in vivo* MRMI data in the placebo treated animals is slightly inconsistent from month 1 to month 2, most likely due to imprecision in the counting method; but at two months the average plaque count is increased from baseline as would be expected. A trend in the direction of a treatment effect was also seen with *ex vivo* MRMI. The *ex vivo* MRMI scans are less noisy than *in vivo* MRMI, and therefore plaque counts tend to be slightly higher which is exactly what is seen in these pilot treatment data. The plaque counts in the Thio-S preparations are considerably higher than counts on MRMI because of the size threshold for MRMI plaque detection. Plaques as small as several micrometers in diameter are detected with Thio-S, whereas plaques must be 35 μm (20 μm for *ex vivo*) to be detected with MRMI. The important feature is the monotonic scaling between MRMI and histological measures. The pilot results for both *in vivo* and *ex vivo* MRMI did not reach statistical significance which might be anticipated given the short treatment time and the small number of animals. But all the trends were concordant – i.e., fewer plaques in treated mice on *in vivo* MRMI, *ex vivo* MRMI and Thio-S.

Acknowledgements

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References

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