Longitudinal Monitoring of Brain Changes in Canines Immunized with Fibrillar or Oligomeric Beta-Amyloid

M-Y. Su[1], L. Vu[1], G. Agrawal[1], K. Head[1], T. Barrett[2], C. Glabe[3], R. Kayed[3], D. Cribbs[3], B. A. Muggenburg[2], E. Head[3], C. W. Cotman[3], and O. Nalcioglu[1]

[1]Tu and Yuan Center for Functional Imaging, University of California, Irvine, CA, United States, [2]Lovelace Respiratory Research Institute, Albuquerque, NM, United States, [3]Institute for Brain Aging and Dementia, University of California, Irvine, CA, United States

Purpose:

As our aging population increases, Alzheimer's disease is rapidly becoming an important healthcare challenge. One of the hallmarks of the disease is the accumulation of beta-amyloid (Aβ) in the brain. Thus, many intervention studies have focused on reducing different conformations of Aβ in the brain. Immunizaing transgenic mice that overexpress human mutant amyloid precursor protein (APP) with fibrillar Aβ, leads to the active production of antibodies against Aβ and clearance or reduction of Aβ in the brain. Further, memory decline appeared to be reduced in immunized mice, suggesting a link between Aβ and cognition. However, in human clinical trials, active immunization led to a subset of patients developing encephalitis, which may have been due to the adjuvant used. Further, transgenic mice passively immunized develop brain microhemorrhages. We tested the hypothesis that active immunization with either fibrillar or oligomeric Aβ would lead to improved cognition and reduced brain pathology in a canine model of human brain aging. Dogs were immunized with fibrillar Aβ (Alum adjuvant), gold-conjugated oligomers or served as controls (adjuvant only, PBS only), which were matched on the basis of cognitive ability. During the following 3 years all dogs underwent extensive testing to evaluate learning and memory ability. Longitudinal MRI studies were performed to monitor changes in the brain at Yr-01, and Yr-2.5 after the baseline. The first follow-up (F/U) MRI was performed 2 weeks after the first vaccination was given to observe any acute changes. The second F/U study was performed 1.5 years later. Changes in the brain (ventricular enlargement and cortical atrophy) were evaluated using co-registered images. The gray matter density in the whole brain between the control and immunization groups were also compared using voxel-based-morphometry (VBM).

Methods:

The study included 29 beagles (8 to 12 years old) with a26 dogs completing 3 MRI studies: baseline, Yr-01 and Yr-2.5. Dogs were separated into 4 groups, immunization with fibrillar Aβ 1-42 (N=9), immunization with gold-conjugated oligomers (N=8), adjuvant-only control (N=6), and a PBS control group (N=3). Animals were immunized subcutaneously in the back of the neck and monitored for adverse reactions. After 2 weeks, animals were boosted with an additional injection. Following the first 2 injections, animals receive a single injection each month. MRI was performed using a GE LX 1.5 Tesla mobile scanner with the knee coil. The animal was anesthetized by inhalation of Isoflurane (1.5-2 %). The protocol included anatomic imaging using a SPGR pulse sequence to acquire a set of 3D images across the whole brain, as well as collecting gradient-echo T2 weighted images and FLAIR (fluid attenuated inversion recovery) images for evaluation of hemorrhage. For each dog the images collected at two F/U studies were co-registered to its baseline (Fig.1) and randomly coded for examination by a neuroradiologist, to compare whether the ventricles in a particular year were larger or smaller, and whether cortical atrophy was more severe in a particular year. We developed methods to use VBM in the dogs and we generated a template, and GM and WM probability maps, as described previously [1]. To increase the subject number the two immunization groups were combined and compared to the 2 combined control groups. A study specific template was generated using all dogs, and the GM maps between these two groups were compared with a threshold level of $p < 0.001$ uncorrected.

Results:

Acute immunization changes were evaluated by comparing the images collected in Yr-01 (2 weeks after the first vaccine was given) to that of baseline. No dogs showed any sign of an adverse effect, no brain hemorrhage or any abnormality was found on MRI, suggesting that the dogs tolerated the vaccine well. Figure 1 shows selected images from one control and one immunized dog. The immunized dog showed clearly visible ventricular enlargement, with subtle cortical atrophy (the only dog determined to have cortical atrophy based on slightly widened sulci). This dog showed the greatest changes among all dogs. Based on a radiologist's visual examination, ventricular enlargement was found in 2/9 control dogs, 2/9 fibrillar Aβ immunized dogs, and 2/8 oligomer Aβ immunized dogs, which was not significantly different across groups. VBM analysis showed that in Yr-01 the immunized dogs had a higher GM density in tissues adjacent to the body of left hippocampus, and the difference became larger in Yr-2.5. The control dogs had a higher GM density in tissues near the posterior part of left hippocampus, which did not increase in Yr-2.5.

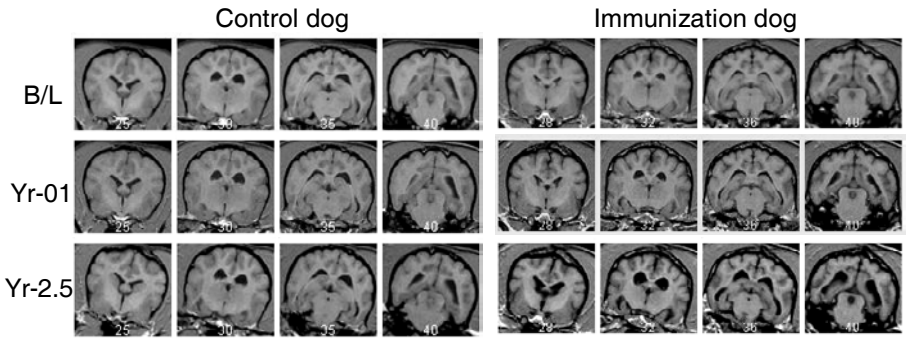

Fig.1 The sample images from one control dog and one immunized dog in 3 MRI studies. In each dog, images were co-registered to its own B/L. The control dog did not show much change. The immunized dog showed a clearly visible ventricular enlargement in Yr-2.5, which was the most noticeable change in all dogs .

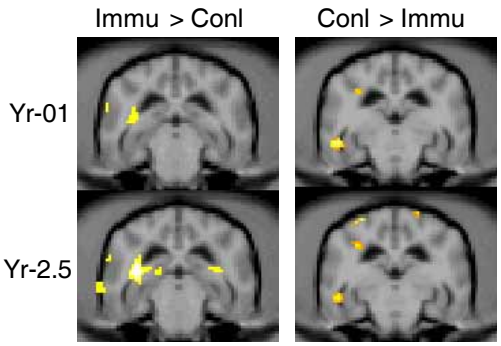

Fig.2 The VBM statistical maps showing the areas where immunized dogs had more GM than the controls, and the controls had more than the immunized dogs.

Discussion:

In this study we investigated brain changes in a longitudinal immunization study in aged dogs using MRI. A visual examination was performed to evaluate ventricular enlargement and to detect changes in cortical atrophy. VBM was used to evaluate gray matter density changes from the whole brain. All dogs tolerated the immunization well without any adverse events. No acute hemorrhage was observed 2 weeks after the first dose of the vaccine was given. Ventricular enlargement is consistently observed in aging dogs [2], and also observed in this study, but it was not more or less extensive between control and immunized groups. Although not the focus of this study, these dogs were euthanized 3 years after the baseline, and the immunized dogs had significantly reduced Aβ in the brain. The analysis is on-going to compare brain pathology with cognitive performance of these dogs to investigate whether the immunotherapy could preserve cognitive ability or slow cognitive decline. Together with these imaging findings, it may be possible to achieve a better understanding of the relationship between cognition, imaging, and neuropathology in monitoring the efficacy of immunotherapy.

References: [1] Tapp et al. Neuroimage 2006; 29:234-244. [2] Su et al. Prog Neuropsychopharmacol Biol Psychiatry. 2005; 29:389-397.

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