EVALUATION OF TRANSLATIONAL DISEASE BIOMARKERS IN THE AGED DOG: THE EFFECTS OF AGE AND BACE INHIBITION ON CSF AMYLOID

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Novel models with greater translational value have been recognized as being necessary for evaluating Alzheimer's disease (AD) therapeutics. Here we describe several features of the aged dog that supports its value as a) a model of AD progression, and b) as a tool for either rapid or longitudinal screening of novel AD therapeutics. The aging dogs models aspects of both the pathophysiology and cognitive decline observed in Alzheimer's disease progression. The current study assessed the effects of age and acute BACE inhibition on CSF amyloid in dogs.

PATHOLOGY

Aged dogs exhibit both early amyloid-β and tau pathologies

Canine amyloid-β (Aβ) protein precursor shows approximately 98% homology to the human sequence and the protein is processed into Aβ isoforms that are analogous in pattern and identical in sequence to that seen in humans (Fig. 1).

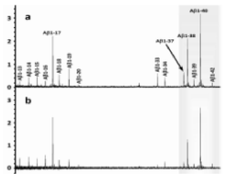


Fig. 1. Representative MOLDI-TOF mass spectra from (a) human and (b) canine CSF samples showing a similar pattern of Aβ isoforms (adapted from Portelius et al., 2010).

Endogenous Aβ is naturally deposited into plaques of the diffuse subtype in aged dogs, which are vulnerable to post translational modification and are fibrillar at the ultrastructural level - cerebral amyloid angiopathy (CAA) is also found in aged dogs. The pattern of Aβ deposition parallels that seen in humans and occurs over a 3- to 4- year window permitting the examination of interventions that may slow or halt deposition (Fig.2).

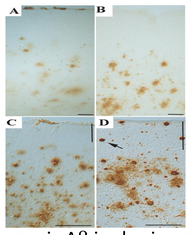


Fig. 2. Cortical Aβ deposition visualized using immunohistochemical staining in (A) cognitively normal aged human (B) cognitively normal aged Beagle, (C) cognitively impaired aged Beagle, and (D) Alzheimer's patient (Adapted from Head et al., 2000).

Oligomeric Aβ is also increased in the aged brain (Fig. 3).

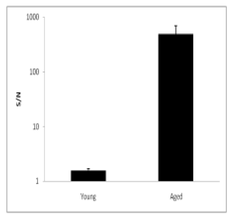


Fig. 3 Signal to noise (S/N) ratio for detecting oligomeric forms of Aβ in brains of young and aged Beagles. Oligomeric Aβ is exponentially higher in aged dogs, as is variability, compared to young dogs using the A4 assay developed by Amorfix (Toronto, Canada).

Intraneuronal hyperphosphoylated tau is also detected in aged dogs that is similar in some respects to the neurofibrillary tangle pathology seen in AD (Fig. 4).

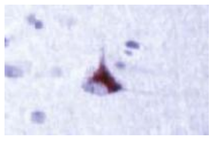
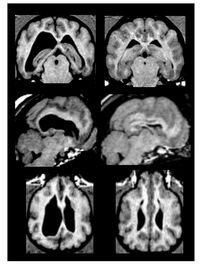


Fig. 4. Immunohistochemical stain with mAb tau AT-8 in aged dog brain in the cerebral cortex (adapted from Papaioannou et al., 2001).

Additional correlates of AD in aged dogs include:

1)Increases in oxidative stress;
2)Neuronal loss and reduced neurogenesis;
3)Reduced markers (e.g. N-acetyl aspartate) of neuronal health;
4) Cholinergic deficits; and
5)Cortical atrophy (Fig. 5).

Fig. 5. Magnetic resonance imaging (MRI) of an aged (left panel) and young (right panel) Beagle. The aged (cognitively impaired) Beagle shows prefrontal and hippocampal atrophy as well as increased ventricular volume compared to the young, cognitively intact, Beagle (see Tapp et al., 2004).



COGNITION

Aged dogs show domain specific and progressive cognitive decline

Aged dogs show cognitive impairment that is correlated with pathology and is domain specific, such as on measures of selective attention (Fig. 6).

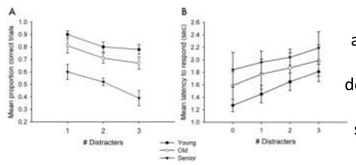


Fig. 6. A selective attention task reveals both age related deficits in performance (A) and processing speed (B) in Beagles.

CSF BIOMARKERS

CSF Aβ42 decreases in Beagles consistent with that seen in conversion to AD (Fig. 7).

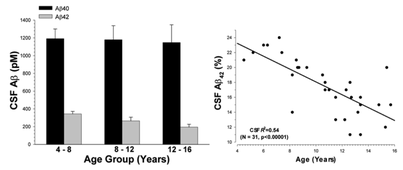


Fig. 7. CSF Aβ42 levels in Beagles decrease with age and is correlated with Aβ deposition (From Head et al., 2010). In humans, decreased CSF Aβ42 is a pathophysiological biomarker of AD evident prior to clinical signs.

Total tau and phospho-tau increases with age in the dog like humans that convert to AD (Fig. 8).

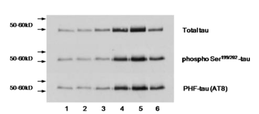


Fig. 8. Measures of tau pathology are increased (Fig. 10) in CSF of aged Beagles (lanes 4-6) compared to young (lanes 1-3) (in collaboration with the National Research Council of Canada; Dr. Balu Chakravarthy).

EFFECT OF BACE INHIBITION AND AGE ON CSF AMYLOID

Methods

Beagle dogs of both sexes were separated into young (2-4 years old, N=15), middle-aged (6-8 years old, N=15), and old (>10 years old, N=15) groups. A cisterna magna sterile puncture under anesthesia was used to obtain CSF, which was then frozen until analysis. Ab40 and Ab42 were measured by ELISA. Each age group was treated with three doses of a proprietary BACE inhibitor (3, 10 and 30 mg/kg; N=5 for a total of 9 groups) two days following the baseline CSF collection. CSF samples were collected 3 hours following dosing. A repeated-measures analysis of variance was used to analyze the data and Tukey's test was used for post-hoc examination of main effects.

Results

CSF Aβ42 is significantly lower with in old dogs

Old dogs showed significantly lower CSF Aβ42 levels compared to both middle-aged [p<0.001] and young [p<0.001] dogs (Fig. 9).

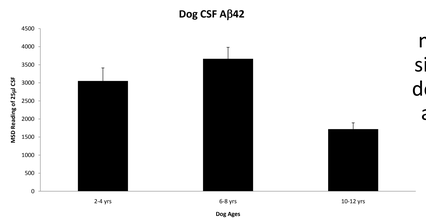


Fig. 9. CSF Aβ42 measured by ELISA was significantly lower in old dogs compared to young and middle-aged dogs. Error bars represent SEM.

BACE inhibition significantly lowered CSF Aβ42

The BACE inhibitor significantly lowered CSF Aβ42 levels compared to baseline in the young and middle age groups [p<0.001 in both cases], but in old dogs (Fig. 10). Percent baseline revealed significantly greater reduction under 30 mg/kg compared to both the 3 [p<0.1] and 10 mg/kg doses [p<0.001].

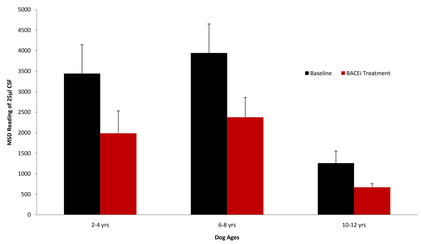


Fig. 10. CSF Aβ42 levels at baseline were significantly lowered by treatment with 30 mg/kg BACE inhibitor in the young and middle aged dogs, but not old dogs. Error bars represent SEM.

CONCLUSIONS

1) **The aged dog is a unique natural model of AD progression.**
2) **Aβ pathology in aged dogs shows similar deposition, identical protein isoforms and oligomeric forms as those seen in humans.**
3) **Additional pathology includes the development of tau-like pathology, increases in oxidative stress, neuronal loss , decreased neurogenesis, reduced neuronal health, cholinergic deficits and brain atrophy.**
4) **The pattern and progression of cognitive decline in translational neuropsychological tests are consistent with that seen in AD conversion.**
5) **Similar to subjects that convert from MCI to AD, Aβ42 in the CSF decreases and phosph-tau increases with advancing age in dogs.**
6) **Here we replicated the findings that CSF Aβ42 decreases with increasing age in dogs. Previous work has linked this decrease in CSF to deposition of amyloid plaques**
7) **We also demonstrated that a BACE inhibitor acutely lowers CSF Aβ42, and that this is least robust in aged dogs, likely due to the relatively lower levels of CSF Aβ42 in this age group.**
8) **Collectively, this supports the use of aging dogs for examining disease modifying AD therapeutics. AD relevant biomarkers such as CSF Aβ42 and tau levels can be used to select target subject groups representing various stages of AD progression, which can then be examined longitudinally for establishing preclinical efficacy.**

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