THE RELIABILITY AND EFFECTS OF AGE ON CSF MEASURES OF β-AMYLOID AND TAU IN BEAGLE DOGS:
IMPLICATIONS FOR A NATURAL ANIMAL MODEL OF ALZHEIMER’S DISEASE PROGRESSION

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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 on CSF amyloid and tau biomarkers across the canine lifespan.

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).

Aged dogs exhibit both early amyloid-β and tau pathologies. Key features include:
- 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).
- Oligomeric Aβ is also increased in the aged brain (Fig. 3).
- Immunohistochemical staining of young and aged Beagles (Oligomeric Aβ is exponentially higher in aged dogs, as is variability, compared to young dogs using the Aβ assay developed by Amorfix (Toronto, Canada).)
- Intranuclear hyperphosphorylated tau is also detected in aged dogs that is similar in some respects to the neurofibrillary tangle pathology seen in AD (Fig. 4).

Additional correlates of AD in aged dogs include:
- Increases in oxidative stress.
- Neuronal loss and reduced neurogenesis.
- Reduced markers (e.g. N-acetyl aspartate) of neuronal health.
- Cholinergic deficits.
- Cortical atrophy (Fig. 5).

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. 2. Cerebral Aβ deposition visualized using immunostaining showing (A) cognitively normal aged human (B) cognitively normal aged Beagle, (C) cognitively impaired Beagle shows preferential and hippocampal atrophy as well as increased ventricular volume compared to the cognitively intact Beagle (see Teppi et al., 2004).

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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 Aβ assay developed by Amorfix (Toronto, Canada).

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

Fig. 4. Immunohistochemical stain with rabbit-anti Aβ 42-6 in aged dog brain in the cerebral cortex (adapted from Pappasouros et al., 2001).

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

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

Fig. 7. CSF Aβ42 levels in Beagles decrease with age and is correlated with Aβ deposition (from Head et al., 2020). In humans, decreased CSF Aβ42 is a pathophysiological biomarker of AD evident prior to clinical signs.

Fig. 8. Measures of tau pathology are increased (Fig. 18) in CSF of aged Beagles (Figures 4-6) compared to young Beagles (Figures 1-3) (in collaboration with the National Research Council of Canada, Dr. Bali Chakravarthy).

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CSF BIOMARKERS

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

Fig. 9. Concentration of Aβ42 and total tau across 4 days in young and aged dogs. The mean within-subject coefficient of variance was 10.9% and 11.3% for Aβ42 and Aβ40, respectively (A) and 9.7% for total tau (B). In all instances no sample differences were found. Error bars represent SEM.

Effect of age on CSF amyloid and total Tau measures

Fig. 10. CSF %Aβ42 and total tau levels across age groups. A) A significant effect of age (F1, 126)=5.471, p=0.002) on %Aβ42 was found, which reflected significantly higher levels in middle aged dogs compared to all other age groups (p<0.05 in all cases). Also, levels in senior dogs were significantly (p<0.05) lower than in old dogs. B) A significant effect of age (F2, 61)=8.0426, p=0.001) on total tau levels was also found, which reflected significantly lower levels in middle aged dogs compared to both old and senior dogs (p<0.05 in both cases). 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 phospho-tau increases with advancing age in dogs.
6) Here we demonstrated acceptable reliability of CSF amyloid and tau measures and replicated the findings that CSF Aβ42 significantly decreases with increasing age in dogs. We also demonstrated CSF %Aβ42 increases significantly from young to middle age, which suggests Aβ42 concentrations may increase to a threshold before deposition occurs.
7) Collectively, this supports the use of aging dogs for examining disease modifying AD therapeutics. AD relevant biomarkers such as CSF Aβ42 and tau levels may 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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