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

C. de Rivera[1,2], B. Chakravarthy[3], S. Kelly[2], J. Baulk[2], M.L. Brooks[2], B. Milgram[1,4], J.A. Araujo[1,5],

[1]Pharmacol, Univ. of Toronto, Toronto, ON, Canada; [2]Vivocore Inc., Toronto, ON, Canada; [3]Human Hlth. Therapeut., Natl. Res. Council, Ottawa, ON, Canada; [4]CanCog Technologies Inc., Toronto, ON, Canada; [5]InterVivo Solutions Inc., Toronto, Canada

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).

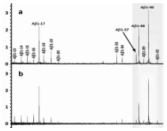


Fig. 1. Representative MOLDI-TOF mass spectra from (a) human and (b) canine CSF samples showing a similar pattern of Aβ isoforms (adapted from Portelius et al., 2010).

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).

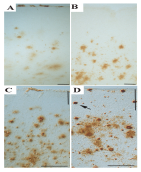


Fig. 2. Cortical Aβ deposition visualized using immunohistochemical staining in (A) cognitively normal aged human (B) cognitively normal aged Beagle, (C) cognitively impaired aged Beagle, and (D) Alzheimer's patient (Adapted from Head et al., 2000).

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

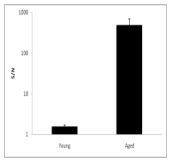


Fig. 3 Signal to noise (S/N) ratio for detecting oligomeric forms of Aβ in brains of young and aged Beagles. Oligomeric Aβ is exponentially higher in aged dogs, as is variability, compared to young dogs using the A4 assay developed by Amorfix (Toronto, Canada).

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).

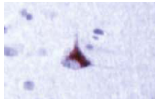
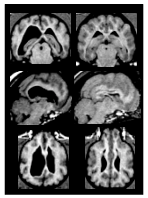


Fig. 4. Immunohistochemical stain with mAb tau AT-8 in aged dog brain in the cerebral cortex (adapted from Papaioannou et al., 2001).

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).

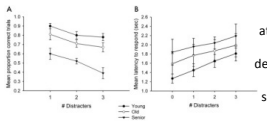


Fig. 6. A selective attention task reveals both age related deficits in performance (A) and processing speed (B) in Beagles.

CSF BIOMARKERS

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

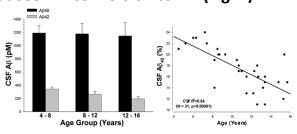


Fig. 7. CSF Aβ42 levels in Beagles decrease with age and is correlated with Aβ deposition (From Head et al., 2010). In humans, decreased CSF Aβ42 is a pathophysiological biomarker of AD evident prior to clinical signs.

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

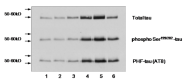


Fig. 8. Measures of tau pathology are increased (Fig. 10) in CSF of aged Beagles (lanes 4-6) compared to young (lanes 1-3) (in collaboration with the National Research Council of Canada; Dr. Balu Chakravarthy).

EFFECT OF AGE ON CSF AMYLOID

Methods

CSF samples from N =130 (age range: 2.0 – 17.3 years) dogs were collected into polypropylene tubes by cysterna magna puncture under short-term anesthesia. Dogs were divided into four age groups (young, n=17, 2.00–2.58 years; middle-age, n=21, 6.33–7.25 years; old, n=57, 8.00–11.92; and senior, n=35, 12.00–17.33 years). Aβ42 and Aβ40 were quantified by ELISA. A subset of the samples (n=64) were analyzed by western blot to semi-quantify total tau levels. To determine reliability, a second sample was collected from a subset of 10 dogs, at the same time of day, four days later. Percent Aβ42 and total tau served as the dependent variable for the age analysis and Aβ42, Aβ40 and total tau levels for determining reliability, both of which were analyzed by ANOVA.

Results

Reliability of CSF amyloid and Total Tau measures

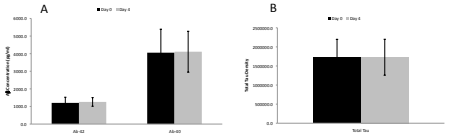


Fig. 9. Concentration of $A\beta_{42}$,$A\beta_{40}$, and total tau across 4 days in young and aged dogs. The mean within-subject coefficient of variance was 10.9% and 11.3% for $A\beta_{42}$ and $A\beta_{40}$, respectively (A) and 9.7% for total tau (B). In all instances no sample differences were found. Error bars represent SEM.

Effect of age on CSF amyloid and tau measures

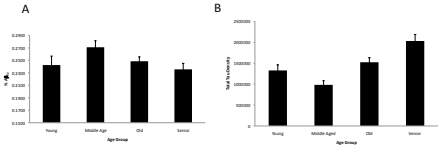


Fig. 10. CSF %Aβ42 and total tau levels across age groups. A) A significant effect of age [F(3, 126)=5.471, p<0.002] on $\%A\beta_{42}$ was found, which reflected significantly higher levels in middle aged dogs compared to all other age groups (p<0.05 in all cases). Also, levels in senior dogs were significantly (p<0.05) lower than in old dogs. B) A significant effect of age [F(2, 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 phosph-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.**
7) **We also demonstrated CSF %Aβ42 increases significantly from young to middle age, which suggests Aβ42 concentrations may increase to a threshold before deposition occurs.**
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 may be used to select target subject groups representing various stages of AD progression, which can then be examined longitudinally for establishing preclinical efficacy.**

Email:josepha@intervivo.com
www.intervivo.com