The aged dog model of Alzheimer's Disease progression: Changes in fluid biomarkers with age and RD2, a novel AD therapeutic

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Introduction

Canine aging is associated with cognitive decline linked to several neuropathological changes that parallel those seen in Alzheimer's disease (AD), including cerebral deposition of amyloid-β. Domain specific cognitive decline is also observed, with short-term working memory and executive function impacted early in canine aging. One contributing factor of AD is neuroinflammation, including activation of microglia and astrocytes, which are also seen in the aged canine brain. In the first study, we sought to investigate whether concentrations of TNFα, IL-2, IL-6 and IL-8 vary by age in cerebrospinal fluid (CSF) along with levels of $A\beta_{40}$ and $A\beta_{42}$. In the second study, we investigated whether a novel AD therapeutic, RD2, would slow down cognitive impairments in aged dogs and whether such changes would be reflected in the following biomarkers: $A\beta_{42}$, total tau, GFAP, NfL and β-synuclein in CSF samples.

Methods

Study 1

CSF was collected from the cisterna magna of 43 dogs in three age groups: young (n = 14, mean age = 2.5 years), middle-aged (n = 13, mean age = 7.7), and senior (n = 15, mean age = 12.3 years).

Concentrations of IL-2, IL-6, IL-8, TNF-α, $A\beta_{40}$, and $A\beta_{42}$ were quantified using a commercially available canine multiplex cytokine kit and analyzed by the MESO QuickPlex SQ. Separate Kruskal-Wallis tests were conducted to determine an effect of age group and post-hoc comparisons were analyzed using Dunn's multiple comparison tests.

Study 2

In the second study, 36 aged beagle dogs (age 9.8 ± 2.5 years) were treated orally for three months with low (3mg/kg/day) or high doses (30mg/kg/day) of RD2 or placebo (N=12 per group). The dogs were tested on the delayed non-match to position (DNMP) and a selective attention task longitudinally, and CSF samples were collected at baseline, every month during the treatment period and during an additional two months after treatment discontinuation. Separate two-way repeated measures analysis of variance tests were conducted to determine the effects of timepoint and dose group. Post-hoc comparisons were analyzed using the Fisher's test.

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Results

Study 1

There was a significant effect of age group on concentrations of IL-2 [$p < 0.05$] and IL-6 [$p < 0.05$]

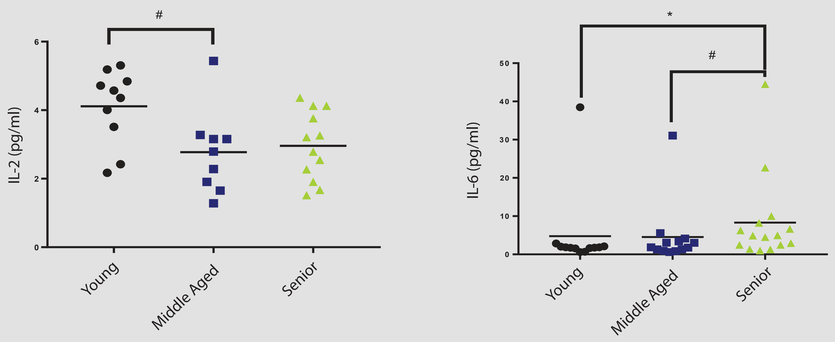

Figure 1

On the left graph, the concentration of IL-2 is significantly lower in the middle-age group compared to the young group. On the right graph, the concentration of IL-6 is significantly higher in senior dogs compared to young (*$p < 0.05$) and there was also a trend for senior dogs to have higher IL-6 levels compared to middle-aged dogs (#$p < 0.07$).

The CSF levels of both $A\beta_{40}$ [$F(2,37) = 12.1587$, $p < 0.0005$)] and $A\beta_{42}$ [$F(2,37) = 6.3943$, $p < 0.005$] significantly increased with age in dogs.

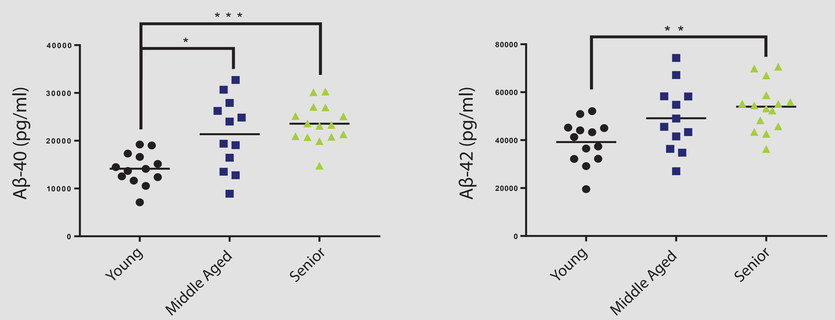

Figure 2

On the left graph, the CSF levels of $A\beta_{40}$ are significantly lower in young dogs compared to both senior (***$p < 0.0005$) and middle-aged dogs (*$p < 0.05$). On the right graph, the CSF levels of $A\beta_{42}$ are significantly higher in senior dogs compared to young dogs (**$p < 0.005$).

Results

Study 2

In study 2, RD2 showed treatment effects on both the DNMP and selective attention test and the changes were sustained even after treatment.

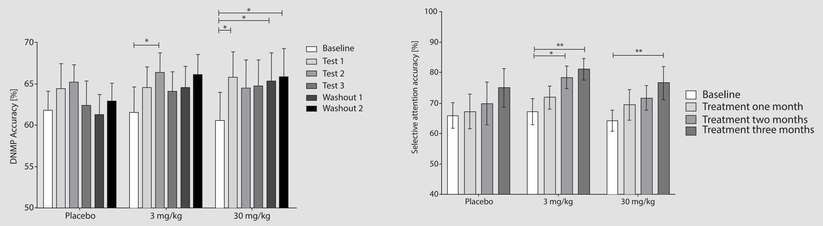

Figure 3

On the left, there was significantly enhanced performance (*$p < 0.05$) after one month of treatment
compared to baseline in the high dose group and after two months treatment in the low dose group
(*$p < 0.05$). The significant treatment effect (*$p < 0.05$) in the high dose group was maintained two months after treatment. On the right, there was enhanced performance on the selective attention task after three months of treatment in both treatment groups (**$p < 0.01$), and in the 3 mg/kg dose group after two months of treatment (*$p < 0.05$).

There was also a significant treatment-dependent CSF tau oligomer decrease at 30 mg/kg RD2 compared to the other treatment groups ($p < 0.05$). There were no significant changes in the other biomarkers.

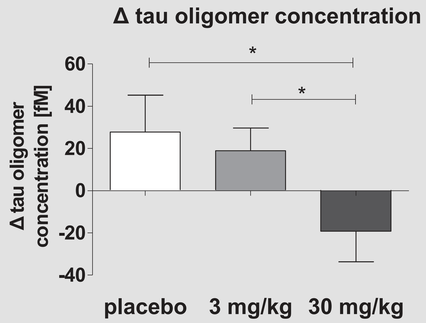

Figure 4

A Mann-Whitney U test revealed a significant difference in tau oligomers between the 30 mg/kg RD2 group and both other treatment groups. *$p < 0.05$ following treatment compared to baseline.

Conclusion

Collectively, these studies further support the use of aged dogs for examining disease-modifying AD therapeutics. AD relevant biomarkers may be used to select target subject groups representing various stages of AD progression, which can then be examined longitudinally for establishing preclinical efficacy.