

Longitudinal Changes in Vascular Function in a Canine Aging Model Measured by Dynamic Contrast Enhanced MRI and the Correlation of Blood-Brain-Barrier Permeability with Beta-Amyloid Angiopathy Assessed by Immunohistochemical Staining

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Purpose: Deposition of beta-amyloid (A-beta) in brain tissue and cerebral blood vessels is the pathological hallmark of Alzheimer’s disease. Evidence is suggesting that the oxidative stress may lead to these pathological changes. The oxidative stress may lead to the misprocessing of amyloid precursor protein (APP), which is cleaved to form A-beta. The deposition of A-beta in vessels (A-beta angiopathy) may have consequences on vascular function. The damaged endothelium may cause disruption of the Blood-Brain-Barrier (BBB), also it may exhibit enhanced vasoconstriction leading to chronic cerebral hypoperfusion. The increased BBB permeability and the decreased vascular volume may be detected by using dynamic contrast enhanced MRI (DCE_MRI). We followed a group of cognitively well-characterized old dogs as a model of human aging for 3 years. Yearly MRI examination was performed to measure the anatomic and vascular changes in the brain. Two types of interventions: antioxidant diet and environmental enrichment, alone or in combination, were studied. At the end of Yr-03, half of dogs were euthanized. The extent of A-beta angiopathy was assessed by immunohistochemical staining, and the results were correlated with BBB permeability index measured by MRI.

Methods: The study was conducted on 48 beagles (9 to 13 years old). The baseline MRI study was carried out, then the dogs were separated into: Group-1(C/C) with normal diet and no enriched environment; Group-2(E/C) with enriched environment and normal diet; Group-3(C/A) with antioxidant diet and no enriched environment; Group-4(E/A) with both antioxidant diet and enriched environment. The enriched environment included regular exercises and daily cognitive testing. One baseline and three follow-up MRI studies were performed. The MRI experiments were performed using a GE 1.5 Tesla mobile scanner with the knee coil. The animal was anesthetized by inhalation of Isoflurane (1.5-2 %). A set of 3D images across the whole brain were acquired using a SPGR pulse sequence. A SE pulse sequence (TR/TE= 133/14 ms) was applied to acquire T1-weighted images before and after injection of Gd-DTPA (0.15 mmol/kg). The enhancement kinetics from frontal, parietal, and occipital regions were measured. The early enhancement ratio (ER, signal enhancement at 30-45 sec/pre-contrast intensity) was used as the vascular volume index, and the late ER at 6.5-7.5 min after contrast injection was an indicator for the contrast leakage through BBB (Fig. 2). The BBB permeability index was defined as the contrast leakage divided by the vascular volume. The changes in vascular volume and permeability indices were followed during the 3 years period. At the end of year-03, 23 dogs were euthanized, and the brains were fixed for A-beta deposition analysis. A-beta angiopathy was assessed using antibody against A-beta 40, which was the dominant type for vascular amyloid. A scale from 0 to 3 was used to describe the extent of A-beta staining on vessels (Fig.1): Type 0- no angiopathy, Type 1- a few scattered vessels, Type 2-several scattered or clustered vessels, and Type 3-widespread array of scattered or clustered vessels.

Results: Table 1 summaries the blood volume and the BBB permeability indices measured from frontal, parietal, and occipital regions of 23 dogs whose A-beta angiopathy data are available. The BBB permeability index is higher in the occipital lobe compared to the parietal lobe (significant p<0.05 in all 4 groups) and the frontal lobe (not reaching significance level). Interestingly, the order was the same in A-beta angiopathy grading (mean value from all 23 dogs was 1.13, 0.96, and 0.78 in occipital, frontal, and parietal lobes, respectively). However, there was no one-to-one correlation between MRI results and immunohistochemical A-beta angiopathy staining data. In contrast to BBB permeability, there were no significant differences in the blood volume between different brain regions or between different intervention groups. Figure 3 shows the longitudinal changes of BBB permeability index measured from the parietal and the occipital lobes during the 3 years. It can be seen that the permeability in the last year (Yr-03) was higher in most groups. In 3 intervention E/C, C/A, and E/A groups, the highest change occurred between Yr-02 and Yr-03, while for the control group it occurred from B/L to Yr-01, suggesting that intervention slowed down the worsening of BBB permeability. All 3 intervention groups also showed a significant blood volume increase in the frontal lobe from B/L to Yr-01, i.e. 1 year after the intervention is initiated, but not in the control.

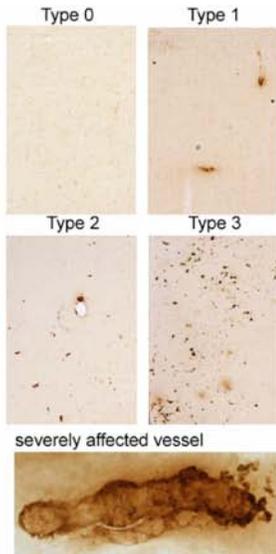


Fig 1: A-beta angiopathy

Table 1: The blood volume (BV), BBB permeability indices, and A-beta staining grades in 4 groups

| | BV-F | BV-P | BV-O | BBB-F | BBB-P | BBB-O | Abeta-F | Abeta-P | Abeta-O |
|-----|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| C/C | 0.24±0.01 | 0.23±0.03 | 0.25±0.04 | 0.47±0.09 | 0.42±0.04 | 0.50±0.08 | 1.33±0.52 | 0.67±0.52 | 1.50±0.84 |
| E/C | 0.21±0.01 | 0.22±0.02 | 0.22±0.01 | 0.48±0.04 | 0.44±0.05 | 0.55±0.12 | 0.80±0.84 | 1.40±1.14 | 1.40±0.55 |
| C/A | 0.21±0.03 | 0.21±0.02 | 0.21±0.01 | 0.47±0.06 | 0.46±0.06 | 0.56±0.12 | 1.17±0.75 | 0.83±0.98 | 1.17±0.75 |
| E/A | 0.21±0.02 | 0.22±0.02 | 0.24±0.03 | 0.45±0.07 | 0.44±0.07 | 0.53±0.10 | 0.50±0.55 | 0.33±0.52 | 0.50±1.22 |

Fig 2: An example of enhancement time course. The early enhancement as vascular index, the late enhancement as BBB leakage, and the ratio of late/early as the BBB permeability index.

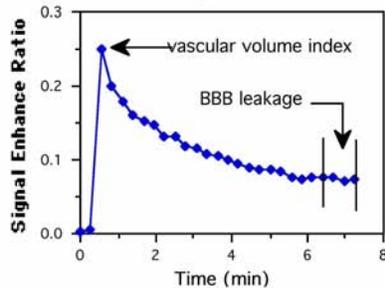
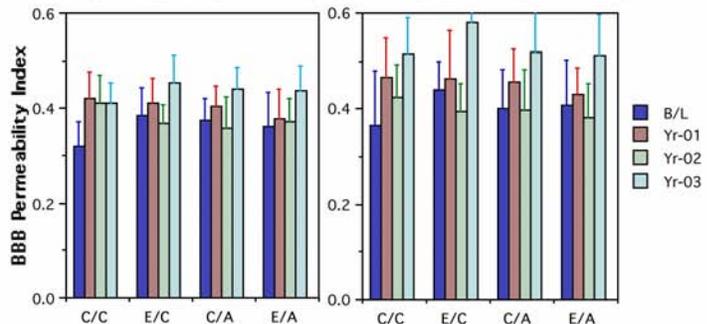


Fig 3: The BBB permeability index measured from the parietal (left) and occipital (right) regions in 4 groups during 3 years, higher in occipital.



Discussion: The canine model is well suited for the assessment of brain aging, including cognitive behavioral performance, neuroimaging, and the final neuropathology. It has been demonstrated that the interventions using antioxidant diet and environmental enrichment could slow down the cognitive decline, especially when they were given in combination [Milgram et al. Neurobiology of Aging 2005; 26:77-90]. In this study we demonstrated that interventions also had some impact on the vascular function. All 3 intervention groups showed increased blood volume in the frontal lobe after one year treatment, and showed delayed BBB permeability worsening for 2 years compared to controls. MRI results demonstrated that BBB permeability was higher in occipital lobe than frontal lobe, which was higher than in parietal lobe, and the order was the same in A-beta angiopathy study. However, there was no significant correlation between MRI and angiopathy data, possible due to difficulty in matching the tissues covered in MRI to that analyzed on immunohistochemical slides.

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