Use of Voxel-based Morphometry Techniques to Assess the Benefits of an Antioxidant Diet in Preventing Cortical Atrophy in a Dog Model of Brain Aging

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Purpose

Aging neuronal tissue is particularly susceptible to oxidative damage due to high oxygen consumption, modest antioxidant defense strategies, and high concentrations of iron and polyunsaturated fatty acids. Prolonged exposure to reactive oxygen species (ROS) causes lipid peroxidation, protein oxidation, and impaired mitochondrial function, which contributes to neuronal dysfunction and cognitive decline. Thus, a potential treatment strategy for age-associated cognitive dysfunction and neurodegeneration is to counteract the damaging effects of ROS and enhance mitochondrial function. The purpose of the present study was to determine if treatment with an antioxidant diet would delay age-related cortical atrophy in beagle dogs. From 1999-2002, aged dogs were fed either a control diet or a diet containing a broad spectrum of antioxidants and mitochondrial co-factors for a 30 month period. T1-weighted anatomical MRI scans were performed annually with a 1.5 T MRI and changes in regional brain volume as a function of diet were examined in the final year (2002) using voxel-based morphometry techniques (VBM) [1,2].

Methods

Thirty-seven beagle dogs (8 to 12 years at baseline) were fed a control (n=16) or an antioxidant diet (n=21) containing enhanced levels of D-L-α-tocopherol acetate, L-carnitine, D-L-α-lipoic acid, ascorbic acid, and 1% of spinach flakes, tomato pomace, grape pomace, carrot granules, and citrus pulp. T_1-weighted images were acquired annually with a GE 1.5T mobile MRI scanner and a quad-knee RF coil (SPGR pulse, NEX = 2; 256 x 256 matrix, 12 cm field of view; repetition time [TR] = 40 msec; echo time [TE] = 9.0 msec; flip angle = 40^0; slice thickness = 1.2 – 1.4 mm; pixel size = 0.47 mm). Differences in regional brain volume in the final year (2002) were assessed with VBM procedures performed using statistical parametric mapping (SPM2). A subject specific template was produced using an iterative process which consisted of averaging AC-PC oriented co-registered images, normalizing the scans with a 12 parameter linear affine transformation and repeating the steps using the template until the best image was attained. Four iterations resulted in a voxel size .5mm X .5mm X .5mm and dimensions of 149 X 197 X 121. All scans were normalized to the template and segmented into GM, WM and CSF. Segmented images were then averaged across all subjects to produce GM, WM and CSF probability maps (figure 1) which were masked to remove undesirable segmentation artifacts. The template and masked probability maps were smoothed with a 1 mm FWHM Gaussian smoothing kernel. Normalized subject scans were then subjected to the optimized VBM protocol [2] using the template and probability maps. GM, WM, and CSF segments produced by the optimized VBM process were visually inspected and masked using the same masking procedure in the production of probability maps. Resultant GM and WM files were smoothed with a 3mm FWHM Gaussian kernel for analysis in SPM2. Statistical inferences were made using an analysis of covariance ($p < .001$) with age at start of intervention as a covariate to compare differences in regional brain volume between antioxidant with non-antioxidant treatment.

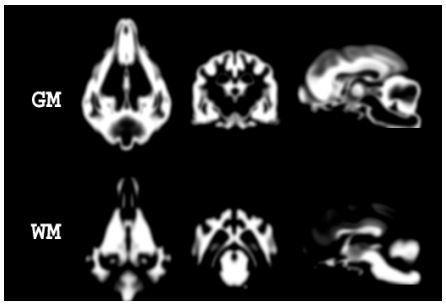

Figure 1. Probability maps for GM and WM in the beagle dog brain.

Results

GM and WM brain volumes that were largest in the antioxidant treated animals relative to the non-antioxidant treated group including age at start of treatment as a covariate are indicated in Table 1. Largest voxel-clusters ($p < .05$ FDR-corrected) are expressed as anatomical landmarks in the canine brain [3]. A sample of GM brain regions that were larger in the antioxidant treated animals that started the intervention at younger ages are shown in figure 1, was observed as a significant age by treatment interaction. Specifically, the thalamus (Th), suprasylvian gyrus (SG), caudate (Cd), lateral gyrus, and cingulate gyrus (CG) were larger in the youngest antioxidant treated subjects.

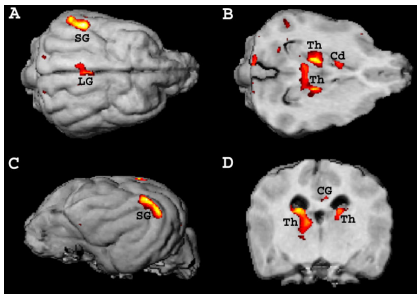

Figure 2. Surface rendering of GM regions in dorsal (A), mid-transaxial (B) lateral (C), and coronal (D) views of the dog brain. Thamalus (Th), cingulate (CG) caudate (Cd), suprasylvian (CG), and lateral gyrus (LG) areas were largest in the antioxidant treated subjects when the diet was started at younger ages.

Cluster Size	Z-stat	GM	Cluster Size	Z-stat	WM
1375	5.48	L- Suprasylvian Gyrus	1135	5.13	L, R-Precommissure septum
650	5.10	L-Cingulate gyrus	1264	4.43	L-Fimbra/Fornix
903	4.58	L-Thalamus		4.23	L-Fornix Column
124	4.24	L-Ectosylvian gyrus	139	4.36	L-Radial Corpus callosum
269	3.96	L-Cerebellum	361	4.27	R-Fascicular subcallosum
149	3.89	L-Corpus Trapezodium	235	4.17	R-Lateral Olfactory stria
264	3.88	L-Lateral Gyrus	356	4.14	R-Lateral Olfactory stria
1242	3.83	R-Thalamus	304	3.91	L-Alveus

Table 1. Individual GM and WM regions that were larger in treated animals versus untreated animals.

Discussion

In the present study, VBM procedures were used to examine changes in GM and WM regional brain volume when a broad-spectrum antioxidant diet was administered to aging beagle dogs over a 30 month period (1999-2002). When VBM analysis was performed on scans acquired in the final year of the study, dogs fed the antioxidant diet had larger GM and WM brain volumes, predominately in the temporal-parietal regions, compared to dogs fed the control diet. The effects of antioxidant diet on brain volume were greatest in the youngest dogs in this study. This suggests that benefits of antioxidants in delaying age-related cortical atrophy are greatest when the intervention is started as early as possible along the aging epoch.

References

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Acknowledgements

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