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Review

Antioxidants in the canine model of human aging ☆

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ABSTRACT

Oxidative damage can lead to neuronal dysfunction in the brain due to modifications to proteins, lipids and DNA/RNA. In both human and canine brain, oxidative damage progressively increases with age. In the Alzheimer's disease (AD) brain, oxidative damage is further exacerbated, possibly due to increased deposition of beta-amyloid (Aβ) peptide in senile plaques. These observations have led to the hypothesis that antioxidants may be beneficial for brain aging and AD. Aged dogs naturally develop AD-like neuropathology (Aβ) and cognitive dysfunction and are a useful animal model in which to test antioxidants. In a longitudinal study of aging beagles, a diet rich in antioxidants improved cognition, maintained cognition and reduced oxidative damage and Aβ pathology in treated animals. These data suggest that antioxidants may be beneficial for human brain aging and for AD, particularly as a preventative intervention. This article is part of a Special Issue entitled: Antioxidants and Antioxidant Treatment in Disease.

1. Introduction

Progressive oxidative damage is a consistent feature of aging [1–4]. The brain is particularly vulnerable to oxidative damage, as it is consumes approximately 20% of the body's total oxygen, has a high content of polyunsaturated fatty acids and has lower levels of endogenous antioxidant activity relative to other tissue [5–7]. Normal metabolic processes result in the release of reactive oxygen species (ROS), which in turn can lead to oxidative damage to proteins, lipids, DNA and RNA [1]. ROS are produced primarily from mitochondria [8], intracellular organelles that are themselves vulnerable to oxidative damage [9]. The combination of mitochondrial dysfunction and production of ROS may be a key contributor to the deleterious effects of aging on the brain [10–18].

Studies of normal human brain aging provide correlative evidence suggesting that oxidative damage plays a role in age-associated cognitive losses. Studies of human autopsy tissue show higher levels of oxidative damage to nucleic acids [19–21], proteins [20,22–25] and lipids [21,25,26] in aged brain as compared to young brain. Mitochondrial function also appears compromised with age in the human brain [3,4,14,27]. In normal aging, mitochondrial respiratory chain activity declines [28], mitochondrial metabolism-associated enzymes such as aconitase decrease [29] and the rate of somatic mitochondrial DNA mutations increases [17,30]. Thus, mitochondrial dysfunction and the production of ROS, combined with lower endogenous antioxidant activity, may lead to increasing oxidative damage to molecules critically important to neuronal function.

2. Oxidative stress and Alzheimer's disease

Oxidative damage may also play a role in age-associated neurodegenerative diseases such as Alzheimer's disease (AD) [31–33]. AD is a progressive neurodegenerative disease that causes dementia in the elderly. AD is characterized by the accumulation of beta-amyloid (Aβ) in extracellular senile plaques and intracellular hyperphosphorylated tau protein in neurofibrillary tangles [34]. Consequently, extensive neuron loss is observed in the AD brain in the cortex and particularly within the hippocampus, a region of the brain involved with memory. AD is associated with further increases in oxidative damage to protein [20,23,24,35–42], lipid [21,26,43–46], DNA [47–49] and RNA [12,50,51] relative to elderly controls. In addition, endogenous antioxidant activity in the AD brain is reduced relative to age-matched controls [20,25,52]. Proteins particularly vulnerable to oxidative damage have been identified by proteomics, with a subset of these proteins putatively involved directly or indirectly in the production and accumulation of AD neuropathology [53]. Mitochondrial dysfunction also occurs in AD, with decreased respiratory chain activity [15,54,55] and increased mitochondrial DNA mutations [56] observed at higher rates when compared to age-matched controls. Further, decreased cytochrome oxidase activity in the posterior cingulate cortex of AD patients is correlated with hypometabolism seen by positron emission studies [57]. A gene array study in the cingulate cortex shows that energy-metabolism related genes decrease

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in AD, with a 65% reduction in expression of mitochondrial electron transport chain genes [58].

Based on correlative human neuropathology studies, antioxidants are predictive of healthy aging, may reduce the risk of developing AD and may improve cognitive function in AD patients. However, studies in humans have shown either a positive effect of antioxidant use on cognition and risk reduction for developing AD [59–61] or no significant effects [62–65]. Few systematic and controlled clinical trials have evaluated the effects of antioxidants on cognition in aged individuals or patients with AD. Intake of vitamin E delays institutionalization in AD patients [66], suggesting some beneficial effects. However, vitamin E alone did not improve cognition in patients with mild cognitive impairment, which is thought to precede AD [67]. Further, in non-demented elderly women, vitamin E treatment was associated with little improvement in cognition [64].

In addition to investigating the effects of cellular antioxidants on cognition and risk of AD, several studies examined the effects of targeted co-factors that improve mitochondrial function, including acetylcarnitine (ALCAR) and lipoic acid (LA). ALCAR and LA may improve mitochondrial function and reduce the production of ROS, thus also reducing oxidative damage to proteins, lipids and DNA/RNA [68]. In studies where ALCAR was administered to patients with moderate to severe AD, either improved cognition and/or slower deterioration was observed [69–72]. In early-onset AD patients (less than 65 years of age), only small cognitive improvements were noted [73], although younger patients with AD (less than 61 years) may also have experienced slowed disease progression [74,75]. When the results of all these studies are combined in a meta-analysis, ALCAR administration in patients with AD was clearly beneficial, particularly with respect to slowing cognitive decline [76]. Further, combining ALCAR with acetylcholinesterase therapy in AD may provide additional benefits [77]. Similar evidence of maintenance of function was observed in an open label study of 9 patients with AD or related dementias receiving 600 mg/day of LA for an average of 337 days [78]. In a larger follow up study of 48 patients for a 48 month treatment period, maintenance of function was also observed [79].

Taken together, studies of dietary or supplemental antioxidant intake in humans reveal variable results and appear far less robustly associated with positive functional outcomes than those reported in the rodent aging literature [9,18,80–87]. Variability in the outcomes of human antioxidant clinical trials outcomes may reflect inconsistencies in the amount of supplements provided, their form and source (e.g. lower AD brain neuropathology is associated with cerebrospinal fluid levels of alpha-tocopherol and not gamma-tocopherol [88]), their duration and regularity of use and challenges in determining the exact background of dietary antioxidants [89]. Interestingly, combinations of antioxidants may be superior to single compound supplementation [90] and dietary intake of antioxidants is superior to supplements in human studies on cognition and risk of developing AD [91,92]. Further, supplementation of elderly women with a combination of vitamins E and C can lead to improved memory [93]. Thus, antioxidants may prove to be more efficacious if administered in combination with other antioxidants (e.g. vitamin C, which helps to recycle Vitamin E) and through diet, rather than as a supplement.

A panel of experts for the Duke Evidence-based Practice Center for the US Department of Health and Human Services recently reviewed the literature and, not surprisingly, reported no consistent or robust evidence to suggest that single or dual antioxidant use is protective against AD [94]. In terms of preventing cognitive decline with aging, vegetable intake was only weakly associated with decreased risk of developing AD, whereas cognitive training was strongly associated with decreased risk. Thus, the role of either dietary or supplemental antioxidants and level of protection against cognitive decline or AD has yet to be clearly established. Additional reasons for the small or negative effects of antioxidants on cognition in the elderly and for treatment of AD [80,95] include the limitations of animal models (primarily rodent) in terms of ability to predict human response. Therefore, it is useful to consider other animal models of human aging and AD, and also to test the potential for combinations of antioxidants/ mitochondrial co-factors to improve cognition and reduce Aβ. Specifically, dogs are frequently used to evaluate safety of drugs and in food metabolism studies given their substantial similarities to humans.

3. Studies in aged dogs

Dogs may be particularly useful in studying human brain aging because they naturally develop cognitive decline with age, accumulate oxidative damage and Aβ protein [96]. In dog brain, oxidative damage to proteins increases with age [97,98] and is associated with reduced endogenous antioxidant enzyme activity or protein levels [97,99–101]. In several studies, a relation between age and increased oxidative damage has been inferred by measuring the amount of end products of lipid peroxidation to predict oxidative damage to lipids. These end products include 4-hydroxynonenal [101–104] and malondialdehyde [97]. Additionally, we and others have reported evidence of increased oxidative damage to DNA or RNA (8OHdG) in aged dog brain [96,104].

Oxidative damage may also be associated with behavioral decline in dogs. Rofina and collaborators examined oxidative end products (lipofuscin-like pigment and protein carbonyls) in aged companion dog brain [98,103,104] and found a correlation between increased oxidative end products and severity of behavior changes due to cognitive dysfunction. Similarly, in our own studies of aging beagles, higher protein oxidative damage (3-nitrotyrosine) and lower endogenous antioxidant capacity (superoxide dismutase and glutathione-S-transferase) are associated with poorer prefrontal-dependent and spatial learning [100]. These correlative studies suggest a link between cognition and progressive oxidative damage in the dog, suggesting their utility in testing antioxidant treatment strategies.

To test the hypothesis that reduced oxidative stress leads to cognitive benefits, we implemented a longitudinal study of aged dogs. In this study, a combination of antioxidants and mitochondrial co-factors was provided in food [105–109]. 48 aged beagles (between ~8 and 12 years) were divided into four groups that were balanced with respect to baseline cognitive ability, sex and age: (1) no behavioral enrichment/control diet group; (2) behavioral enrichment/control diet; (3) no behavioral enrichment/antioxidant diet; and (4) combined behavioral enrichment and antioxidant diet. In a subset of experiments, an additional 17 young beagles (<5 years of age) were included for comparison to aged dogs. Young dogs were all placed in the behavioral enrichment condition, with half provided with the antioxidant diet (i.e. similar to groups 2 and 4).

Three unique features of the experiment included: 1) a combination of antioxidants and mitochondrial co-factors; 2) incorporation of all antioxidants and mitochondrial co-factors into food and; 3) evaluation of dietary treatments in combination with behavioral enrichment. An antioxidant-enriched dog diet was formulated to include a broad spectrum of antioxidants and two mitochondrial co-factors [108]. Based on an average weight of 10 kg per animal, the daily doses for each compound were 800 IU or 210 mg/day (21 mg/kg/day) of vitamin E, 16 mg/day (1.6 mg/kg/day) of vitamin C, 52 mg/day (5.2 mg/kg/day) of carnitine and 26 mg/day (2.6 mg/kg/day) of lipoic acid. Fruits and vegetables were also incorporated at a 1 to 1 exchange ratio for corn, resulting in 1% inclusions (dehydrated) of each of the following: spinach flakes, tomato pomace, grape pomace, carrot granules and citrus pulp. This was equivalent to raising fruits and vegetable intake from 3 servings per day to 5–6 servings per day based upon ORAC values [110]. Additionally, vitamin E was increased by ~75% in dogs treated with the antioxidant diet [111]. The behavioral enrichment condition consisted of additional cognitive experience (20–30 min/day, 5 days/week), an enriched sensory environment (housing with a kennelmate, weekly rotation of play toys in kennel) and physical exercise (two 20 min outdoor walks/week) [108].

Dogs were evaluated over a 2.8 year period to evaluate short term and chronic treatment effects. Treatment with the antioxidant diet leads to cognitive improvements in learning within two weeks, with aged animals showing significant improvements in spatial attention (landmark task) [111]. Subsequent testing of animals with a more difficult complex learning task (oddity discrimination) also revealed benefits of the diet [105]. With antioxidant treatment, visual discrimination improved and reversal (frontal function) learning ability was maintained over time while untreated animals showed a progressive decline [108]. This was despite the fact that for each time point where discrimination learning was re-administered, the task was made more difficult (harder to distinguish objects) to prevent a practice effect. Thus the progressive increase in error scores over time in untreated dogs reflects both increased task difficulty and possibly, longitudinal aging effects. Interestingly, the dogs fed an antioxidant diet benefited from behavioral enrichment, in that cognitive scores of aged dogs receiving both treatments were superior to either treatment alone [107,108]. For example, in singly treated animals spatial memory showed a trend toward improvement, reaching statistical significance only after long-term treatment (>2 years) with a combination of both the antioxidant diet and behavioral enrichment [109]. The antioxidant diet selectively repaired an aging deficit, in that cognitive scores from young dogs treated with the antioxidant diet did not differ from those of young dogs fed control diet [112].

Neurobiological studies showed reduced oxidative damage and increased endogenous antioxidant activity in antioxidant-fed dogs, particularly among animals receiving the combination of antioxidants and behavioral enrichment [100]. Interestingly, the antioxidant diet increased the levels of glutathione suggesting a possible involvement of a possible vitagene network that might account for the increased expression of antioxidant molecules and growth proteins [113,114]. Given that the diet provided to the dogs also included acetylcarnitine, resulting in increased levels of HO-1 also support the possibility that vitagene networks are engaged [113].

Mitochondrial function was significantly improved in the antioxidant fed dogs and not in behaviorally enriched dogs [115]. Interestingly, behavioral enrichment but not the antioxidant diet protected against neuron loss in the hilus of the dog hippocampus [116]. Further, brain derived neurotrophic factor mRNA increased in aged dogs provided with the combination treatment [117]. These results suggest that cognitive benefits of antioxidants can be further enhanced with the addition of behavioral enrichment, perhaps due to different yet synergistic mechanisms of action in the brain, including reduced oxidative damage and maintenance of neuron health. In addition to brain, however, peripheral benefits were also seen, including less cellular degeneration in the inner ear [118].

Interestingly, in a recent study of aged dogs, the formulation of the diet was modified to compare only the mitochondrial co-factors used in this previous study and effects on cognition [119]. Aged dogs were treated with lipoic acid, ALCAR or the combination and tested with spatial learning and discrimination/reversal tasks. When these compounds were included with a broader spectrum of antioxidants described above, no cognitive benefits were observed when evaluated singly or in combination. Additionally, protein carbonyl accumulation in the plasma of treated dogs was increased. Increased oxidative damage may reflect either higher doses of the mitochondrial co-factors used in this study or increased oxidative stress resulting from not counterbalancing mitochondrial cofactors with cellular antioxidants. Consistent with this explanation, another smaller study demonstrated improved short term memory in aged beagles treated with a combination of phosphatidylserine, *Ginkgo biloba*, vitamin E and pyridoxine [120].

4. Summary

Using the canine model of aging, we show that providing a broad spectrum of cellular antioxidants and mitochondrial co-factors within a specially-formulated food leads to significant benefits to cognition and maintenance of function at doses that were well within those used in human clinical trials. In addition, combining an antioxidant enriched diet with cognitive training, physical exercise and social enrichment provides additional benefits to cognition. Future studies in humans may be more efficacious if combinations of antioxidants are evaluated in parallel with additional lifestyle improvements (e.g. social engagement, cognitive training and physical exercise).

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