Strategies for improving cognition with aging: insights from a longitudinal study of antioxidant and behavioral enrichment in canines

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Abstract Studies in humans suggest that lifestyle factors can have a beneficial impact on the risk for developing cognitive decline and dementia with age. There is growing evidence that maintaining a physically and intellectually active lifestyle can positively impact cognitive ability in older individuals. Dietary factors, such as the intake of antioxidants, may also prevent age-related cognitive decline. However, studies in humans are challenging; many variables cannot be controlled, making it difficult for researchers to determine the exact types and quantities of enrichment and dietary factors necessary for positive effects on cognition. Studies in animal models of human aging allow researchers to precisely control such variables, and can be used to assess the mechanisms and molecular pathways underlying any positive effects. Here we review the results of an intervention study using a canine model of human aging. The study was unique in that it compared the effects of dietary antioxidant supplementation alone and in combination with be-

havioral enrichment. We found that both interventions lead to improvements in cognitive ability in aged dogs; however, combining the treatments preserved cognition to a greater extent than either treatment alone. Overall, the results suggest that antioxidant supplementation and behavioral enrichment target separate yet complementary molecular pathways to improve cognition, and support the idea that combinations of treatments to improve cognition and slow brain aging will produce greater benefits than single interventions.

Keywords Beta-amyloid · Dog · Mitochondrial cofactors · Neurogenesis · Oxidative stress · Proteomics

Introduction

A goal of aging research is to identify ways in which to promote the preservation of cognitive function and healthy brain aging. With increasing age, individuals become more vulnerable to neurodegenerative diseases such as Alzheimer's disease (AD). Encouraging beneficial lifestyle choices is a promising approach to reducing the risk for developing neurodegenerative diseases. For example, maintaining or increasing physical, social and cognitively engaging activities and levels of dietary antioxidant intake are relatively simple modifications that can be made on a daily basis. We suggest here, based on studies in human populations and from a treatment study in a canine

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model of human aging, that combining dietary and behavioral enrichment approaches may have striking effects on cognition and neuropathology.

Physical activity and cognitive stimulation are linked to reduced risk for pathological aging in humans

There are a number of human epidemiological studies that suggest maintaining an active lifestyle can protect against pathological aging (Friedland et al. 2001; Kramer et al. 2004; Cotman et al. 2007). For example, participation in leisure activities (Scarmeas et al. 2001; Verghese 2003; Rovio et al. 2005) reduces the risk for developing AD. Engaging in physical activities leads to improved or maintained cognitive function (Laurin et al. 2001; Colcombe and Kramer 2003; Kramer et al. 2003; Larson et al. 2006; Wang et al. 2006), with lower levels of physical activity associated with a higher risk of developing AD or vascular dementia (Ravaglia et al. 2007). Further, intervention studies demonstrate that individuals with AD who exercise show improved activities of daily living and a slower rate of decline on cognitive tests compared with non-exercisers (Teri et al. 2003; Stevens and Killeen 2006).

Observational studies report a link between cognitive stimulation and healthy brain aging; in systematic and controlled experiments using cognitive training interventions, significant long lasting cognitive improvements are observed (Yesavage 1985; Ball et al. 2002; also see review by Acevedo and Loewenstein 2007). For example, in a series of studies by the ACTIVE (Advanced Cognitive Training for Independent and Vital Elderly) study group, older-independent-living adults were provided with one of four treatment conditions: three groups received ten training sessions either for memory, reasoning or speed of processing. A fourth group served as an untreated control sample. In the first of two reports by Willis and colleagues, Ball et al. (2002) showed that each intervention led to immediate improvements in function on tasks specific to that cognitive domain. Further, these enhancements were maintained for over 2 years with booster interventions (Ball et al. 2002). When individuals were followed across an additional 3 years (for a total of 5 years), those receiving reasoning training had less difficulty with activities of daily

living, although the same effect was not seen in the memory or speed of processing training groups (Willis et al. 2006). Additional lifestyle factors, such as a higher level of education or more intellectually challenging occupations, may provide similar life-long behavioral enrichment (see Kramer et al. 2004 for an excellent review; Potter et al. 2007). Overall, observational and intervention studies support a link between physical activity, intellectual stimulation (either by activities, education or occupation) and successful brain aging.

Antioxidants and healthy aging

In contrast to the relatively consistent effects of behavioral enrichment and lifestyle parameters that engage physical and cognitive activities, results from studies on the use of antioxidants in human observational studies are less uniform. While some studies have shown a positive effect of antioxidant supplementation on cognition and risk reduction for developing AD (Engelhart et al. 2002; Morris 2002), other studies do not report significant effects (Masaki et al. 2000; Luchsinger et al. 2003). Difficulties in interpreting human observational studies stem from inconsistencies in the amount of supplements taken, their form and source, their duration and regularity of use, and from the challenges of determining the exact dietary intake of antioxidants. For example, in one study, combinations of antioxidants were superior to single supplementation (Zandi et al. 2004) and dietary intake of antioxidants was superior compared with supplements (i.e., tablets) (Morris et al. 2002).

Frequent consumption of fruits and vegetables, in a recent Three-City cohort study in Bordeaux, Dijon and Montpellier (France), confirms that dietary antioxidants are associated with a reduced risk of developing dementia (Barberger-Gateau et al. 2007). Recent reviews of the human epidemiological literature have emphasized that antioxidants may be one of the most promising treatments for preventing AD (Rutten et al. 2002). Vitamin E has been shown to delay institutionalization in AD patients, suggesting some beneficial effects (Sano et al. 1997). However, vitamin E alone did not improve cognition in patients with mild cognitive impairment, which is thought to be a precursor to AD (Petersen et al. 2005). In normally aging individuals, vitamin E alone has little benefit in

elderly women (Kang et al. 2006); however, one study showed that supplementation with a combination of vitamins E and C led to improved cognition (Chandra 2001). Overall, it is clear that systematic clinical trials evaluating the effectiveness of antioxidants are urgently needed.

Why study aging in dogs?

In the meantime, using animal models to study the effectiveness of antioxidants offers a major advantage in our ability to control many of the experimental variables that can contribute to differences in human observational studies. Animal studies further allow the researcher to understand the brain mechanisms and molecular cascades that might be critical for beneficial treatment effects. However, there are few studies in animal models that develop similar types of brain pathology as the human and that can be studied for extended periods of time. Further, studies in animal models rarely evaluate the effects of combinatorial interventions. For example, can the benefits of an antioxidant diet be enhanced by inclusion of behavioral enrichment? For these reasons, we have been using a canine model of human aging.

Compared with rodent models of aging, the relatively long life span of dogs allows researchers to assess the long-term effects of interventions for brain aging and dementia. Practically, dogs are readily available for research purposes, easy to house and handle and do not require food deprivation as motivation for responding on food-based tasks. Over the past 15 years, our research group has developed an extensive battery of tasks to assess a wide range of cognitive functions in the dog, including spatial and non-spatial learning and memory ability, concept learning and executive function.

The application of these tasks in the canine model has revealed that, just as in humans, the deficits observed in aged dogs depend on the type of cognitive domain assessed, the learning strategy that is engaged by a particular task, and the previous experiences of individual animals. For instance, deficits in spatial memory begin in middle age, in some dogs as young as 6 years old (Studzinski et al. 2006). By contrast, simple spatial discrimination learning remains intact even in senior dogs as old as 12 or more years of age (Christie et al. 2005). This is the case for egocentric

spatial discrimination problems; that is, when dogs must choose one of two identical objects based on their spatial position relative to their own body placement to obtain a food reward. When dogs must choose an object based on its position relative to an external, or allocentric, cue, age-related impairments are evident starting at 8 years of age (Christie et al. 2005). Also like humans, aged dogs show deficits in executive functions thought to rely on frontal cortical regions. For example, when inhibiting responses to a previously rewarded stimulus is required to obtain reward, such as in a reversal-learning task, dogs show impairments beginning at 8 years of age (Tapp et al. 2003).

Dogs also show changes in the types of learning strategies they use with age. For example, old dogs tend to use an associative strategy to solve an oddity discrimination task, while young dogs will use a cognitive strategy to solve the same problem (Milgram et al. 2002). In the oddity task, animals must learn to choose an odd object amongst three objects, two of which are identical. The task is made more difficult by increasing the similarity of the food-rewarded odd object to the non-rewarded objects. Aged dogs will show progressively more errors on each successive task as the appearance of the objects is made more similar, which is consistent with their learning to associate the correct object with reward through repeated pairing of the two. By contrast, young dogs do not show significant increases in errors as the task is made more difficult and, in fact, will sometimes learn each successive task more rapidly, suggesting that they learn the general strategy of responding to the odd object (Milgram et al. 2002).

The extent of age-related decline on these tasks depends on the previous experiences of individual animals. Prior cognitive test experience improves learning in dogs, and the beneficial effects of cognitive training vary as a function of age, with young animals showing greater improvement than old (Milgram 2003). In other words, dogs are able to learn from experience, suggesting that they are suitable models for assessing the mechanisms underlying the beneficial effects of education and intellectual stimulation in humans, as described above.

In addition to the parallels observed in age-related canine and human cognitive dysfunction, aged dogs exhibit many features of normal and pathological brain aging that develop in humans. Several studies have

shown that aged dogs naturally develop diffuse plaques that contain beta-amyloid (A β) peptide similar to those observed in humans with mild cognitive impairment and AD (Wisniewski et al. 1990; Cummings et al. 1993; Hou et al. 1997; Head et al. 2000). Further, dogs and humans express the same 1–42 amino acid sequence A β peptide (Selkoe et al. 1987; Johnstone et al. 1991). Also like humans, some cortical regions in the dog are more vulnerable to A β accumulation than others; the prefrontal cortex shows A β plaque pathology in dogs as young as 8 years old (Head et al. 2000). Several correlation studies support a link between A β accumulation and cognitive deficits in aged dogs (Cummings et al. 1996; Colle et al. 2000; Head et al. 2000; Pugliese et al. 2006; Rofina et al. 2006), although the results are somewhat mixed, as are the results from human studies.

Cortical atrophy and increased ventricular volume are observed in the aged dog brain (Su et al. 1998; Tapp et al. 2004; Kimotsuki et al. 2005) and may underlie some of the cognitive impairments observed in old dogs. In particular, atrophy in frontal regions may contribute to executive dysfunction (Tapp et al. 2004). Recent evidence suggests that cognitive dysfunction is also related to a reduction in neurogenesis in the dentate gyrus of aged dogs (Siwak-Tapp et al. 2007). Siwak-Tapp et al. (2007) observed that cell genesis and neurogenesis are decreased in the subgranular zone/granule cell layer of the hippocampus in old dogs compared with young dogs, and that the number of new neurons in this region correlates with performance on spatial memory and discrimination learning tasks. Recent advances in the availability of canine gene sequence information and commercial microarrays are also beginning to expand our understanding of the mechanisms underlying cognitive decline in the aged canine. Geriatric dogs have increased expression of genes associated with inflammation, stress response and calcium homeostasis along with decreased expression of genes associated with neuropeptide signalling and synaptic transmission (Swanson et al. 2007).

The canine brain also progressively accumulates oxidative damage with age. Aged dogs show damage to protein carbonyl groups in the brain (Head et al. 2002; Skoumalova et al. 2003), in parallel with a reduction in endogenous antioxidant enzymes, including glutamine synthetase and superoxide dismutase (Kiatipattanasakul et al. 1997; Head et al. 2002). Several studies report evidence of increased oxidative

damage to lipids (Papaioannou et al. 2001; Head et al. 2002; Rofina et al. 2004, 2006) and DNA/RNA (Rofina et al. 2006) in the aged canine brain. These findings, along with the observation that the canine's absorption of dietary nutrients is similar to humans, were the main starting points for conducting a long-term dietary antioxidant intervention study using the canine model of human aging.

A longitudinal study of antioxidants and behavioral enrichment in aged canines

We hypothesized that long-term administration of a diet enriched with antioxidants and a behavioral enrichment paradigm would positively affect cognitive function, and that the combination of these two treatments would be more effective at preventing age-related cognitive decline than either alone. We further hypothesized that dogs receiving the antioxidant diet, either alone or in combination with behavioral enrichment, would show decreased age-related brain pathology.

To test these hypotheses, 24 dogs (mean age = 10.7 years at study start) were divided into four cognitively equivalent groups following baseline testing as follows; $n=6$ animals received regular dog food and no behavioral enrichment (control/control group), $n=6$ animals received the antioxidant-enriched diet and no behavioral enrichment (AOX/control group), $n=6$ animals received regular dog food and behavioral enrichment (control/BEH group), and $n=6$ animals received both the antioxidant-enriched diet and behavioral enrichment (AOX/BEH group). Dogs remained in these treatment groups over the entire course of the study, which lasted approximately 2.5 years.

The composition of the antioxidant diet has been described in detail elsewhere (Zicker 2005). Briefly, the diet contained a broad spectrum of antioxidants, including α -tocopherol, vitamin C and fruit and vegetable extracts, along with two mitochondrial cofactors thought to improve mitochondrial function, DL-lipoic acid and L-carnitine. The behavioral enrichment paradigm included cognitive, social and physical stimulation. Cognitive enrichment was provided by administering learning problems to the dogs five-times per week, along with new toys in their home cages. Social enrichment included housing animals with kennel-mates as opposed to housing them singly.

Walking dogs on a leash for at least 20 min, twice per week, provided physical enrichment.

Treatment with the antioxidant-enriched diet produced rapid improvements in learning within 2 weeks. Aged dogs showed immediate improvements in their acquisition of a spatial attention task compared with dogs fed the regular dog food diet (Milgram et al. 2002), and the positive effects of the antioxidant diet on cognition were maintained over the course of the study (Milgram et al. 2005). After prolonged administration of the enriched diet, similar positive effects were found on more complex tasks, such as the oddity discrimination task (Milgram et al. 2002). In this task, animals must learn a concept of choosing the odd object amongst three objects, two of which are identical. Aged dogs are normally impaired on this task, especially as the food-rewarded odd object is made to appear more similar to the non-rewarded objects (Milgram et al. 2002). Old dogs receiving the antioxidant diet showed much improved learning on the task, with error scores comparable with those obtained by young animals (Milgram et al. 2002). Further, the positive effects of the diet were specific to aged animals; young dogs receiving the antioxidant diet showed no benefit on oddity learning compared with young dogs receiving the control diet (Milgram et al. 2002). This suggests that, in young dogs, oxidative stress levels are not high enough to induce substantial neuronal dysfunction and cognitive impairment.

As hypothesized, combined treatment with an antioxidant-fortified diet and behavioral enrichment produced even more pronounced effects on cognition than either treatment alone (Milgram et al. 2004, 2005). Dogs in the AOX/BEH group made significantly fewer errors in learning a size reversal task compared with dogs in the AOX/control, control/BEH and control/control groups (Milgram et al. 2004), suggesting that frontal function was improved by the combination treatment. Further, our findings suggest that the combination treatment was able to maintain cognitive ability in old dogs over the 2.5 years that the study was conducted (Milgram et al. 2005). Old animals that received the control diet and no behavioral enrichment showed a progressive decline in discrimination and reversal learning, while those receiving the enriched diet and/or behavioral enrichment showed fewer failures and a maintenance of learning abilities over the course of the study (Milgram et al. 2005).

Treatment effects on A β pathology and neuron loss

The possible neurobiological mechanisms underlying the beneficial effects of behavioral enrichment (social, physical and intellectual) are actively being studied in animal models. In rodents, improved behavioral function as a consequence of environmental enrichment or physical exercise may be mediated by increased neurogenesis (van Praag et al. 2000, 2005), increased availability of brain growth factors (Cotman and Neepser 1996; Cotman and Berchtold 2002), and possibly by reductions in brain pathology, such as A β accumulation typically seen in patients with AD (Adlard et al. 2005; Lazarov et al. 2005). We have been characterizing the possible mechanisms underlying the cognitive improving effects of the antioxidant diet and behavioral enrichment in canines and have found some interesting dissociations between the two treatments.

One of the first types of pathology measured in the treated and untreated aged canines was the extent of A β accumulation. Antioxidant diet-fed animals, but not behaviorally enriched animals, showed a significant reduction in A β plaque load (Pop et al. 2003). A β deposition was reduced by 27–84% in the parietal, entorhinal, and occipital cortices, but not in the prefrontal cortex. In contrast, age-dependent neuron loss in the hilus of the hippocampus of canines was reduced in behaviorally enriched animals but not in those receiving the antioxidant diet (Siwak-Tapp et al. 2006). Higher hilar neuron number in behaviorally enriched animals, however, was not linked to increased neurogenesis (Siwak-Tapp et al. 2006), suggesting that behavioral enrichment protected the hippocampus from neuron loss but did not increase the proliferation of new neurons.

Treatment effects on protein expression, oxidation and cognition: a proteomics approach

As described above, oxidative stress plays a significant role in the mechanisms leading to the development of cognitive dysfunction in the aging human as well as in various animal models (Markesbery 1997; Butterfield and Kanski 2001), and the use of antioxidants and related compounds is successful in delaying and/or reducing age-related cognitive decline (Joseph et al. 1998; Farr et al. 2003). Progressive accumulation of

oxidative damage and a parallel reduction in endogenous protective antioxidants is observed in the aging canine (Head et al. 2002). A β plays a significant role in the mechanism of oxidative stress as observed in AD (Hensley et al. 1996; Butterfield and Lauderback 2002), and since the aging canine accumulates diffuse plaques that contain A β peptide similar to those observed in humans, we believe that similar mechanisms may underlie cognitive dysfunction in the aging canine.

The combined treatment group showed additional changes in the brain distinct from either treatment alone, as was shown in a comprehensive proteomics study of the parietal cortex of treated animals (Opii et al. 2008). For example, there was a significant decrease in the levels of several toxic oxidative stress biomarkers (protein carbonyls and 3-nitrotyrosine) in animals receiving both the antioxidant diet and behavioural enrichment compared with groups receiving the single treatments. A global proteomic analysis of the parietal cortex of dogs receiving the combination treatment showed a significant increase in the expression and enzymatic activities of proteins involved in energy metabolism, antioxidant systems, and in the maintenance and stabilization of cell structure, all of which play a significant role in memory and cognitive function (Opii et al. 2008). For example, the expression

levels of energy metabolism related proteins, i.e., Cu/Zn superoxide dismutase, fructose-bisphosphate aldolase C, creatine kinase, glutamate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, were significantly increased in the group of animals receiving the combination treatment when compared with controls. In addition, the combination treatment provided protection against oxidative damage to key proteins, i.e., glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, α -enolase, and glutathione-S-transferase (GST), as evident from the reduced levels of oxidation assessed by redox proteomics. Further, this was paralleled by a significant increase in activity of endogenous antioxidant activities of superoxide dismutase and glutathione-S-transferase and a significant increase in the protein levels of heme oxygenase-1 (HO-1), an important component of the neuronal defence system.

More importantly, the reduction in oxidative damage coupled with increased expression and activity of protective proteins and enzymes was found to correlate with improvements in cognitive function (Opii et al. 2008). Briefly, the Cu/Zn superoxide dismutase (SOD) protein level was negatively correlated with error scores on a black/white reversal task and on a spatial memory task, i.e., higher antioxidant protein level was associated with improved cognition.

Fig. 1 Long-term administration of an antioxidant-enriched diet or a program of behavioral enrichment in a canine model of aging activates separate and complementary molecular pathways. Dogs who received an antioxidant-enriched diet or behavioral enrichment showed improved cognitive function. However, only animals fed the antioxidant diet showed decreased A β plaque load, while only enriched animals showed decreased neuronal loss. Animals receiving both treatments

showed the greatest preservation of cognitive function over the course of the study, and had lower brain levels of oxidative damage and increased levels of protective antioxidant enzymes. Although additional mechanisms are most likely involved in the protective effects of these treatments, our results suggest that combining interventions will have a greater impact on preventing age-related cognitive decline than single treatments

In addition, higher error scores on tests of black/white discrimination, black/white reversal and spatial memory were associated with higher levels of oxidative damage as measured by the levels of 3-nitrotyrosine (3-NT). Also, higher levels of antioxidant enzyme activity (SOD, GST) or higher protein levels of HO-1 were generally associated with lower error scores on all tasks. These findings suggest that the combination treatment led to improved cognition by reducing the level of oxidative damage while also increasing the expression and activity of key proteins and enzymes.

Discussion

Studies in humans suggest that lifestyle factors can have a beneficial impact on the risk for developing cognitive decline and dementia with age. There is growing evidence that maintaining a physically and intellectually active lifestyle can positively impact cognitive ability in older individuals, and there is some evidence that dietary factors, in particular the intake of antioxidants, may have a protective effect as well. These studies are challenging, however, in that many variables cannot be controlled, making it difficult for researchers to determine the exact types and quantities of enrichment and dietary factors that may impact age-related cognitive decline, as well as the mechanisms underlying their positive effects.

The canine intervention study reviewed presently was unique in that it compared the effects of dietary antioxidant supplementation alone and in combination with behavioral enrichment. We found that both interventions alone lead to improvements in cognitive ability in aged dogs; however, combining both treatments further preserved cognition. This observation, and the differential brain changes we observed in post mortem analyses, suggest that antioxidant supplementation and behavioral enrichment target separate yet complementary molecular pathways to improve cognition (Fig. 1). Treatment with an antioxidant-enriched diet, and not behavioral enrichment, decreased A β plaque load. Engaging animals in a physical, cognitive and social enrichment paradigm preserved neuron number in the hippocampus, while the antioxidant diet appeared to have no effect on age-related cell loss. Further, both interventions in combination were necessary to significantly impact brain oxidation and endogenous antioxidant enzyme levels.

Our results support the idea that combination of treatments to improve cognition and slow brain aging will produce greater benefits than single interventions. Indeed, research in humans seems to be moving in this direction. For example, a new study has been initiated, the Fit Bodies, Fit Minds project that will assess whether physical activity alone or in combination with cognitive training improves cognition in 65–75 year old non-demented individuals (O'Dwyer et al. 2007). Overall, the results of our study using a canine model of human brain aging suggest that modifying environmental factors and diet has a significant impact on maintaining brain function and health.

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