Brain aging in the canine: a diet enriched in antioxidants reduces cognitive dysfunction

Carl W. Cotman a,∗, Elizabeth Head a, Bruce A. Muggenburg b, S. Zicker c, Norton W. Milgram d

a Institute for Brain Aging and Dementia, University of California, 1226 Gillespie Neuroscience Research Facility, Irvine, CA 92697-4540, USA
b Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr. SE, Albuquerque, NM 87108, USA
c Science & Technology Center, Hill’s Pet Nutrition, Inc., P.O. Box 1658, Topeka, KS 66601, USA
d Life Science Division, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, Ont., Canada M1C 1A4

Received 26 February 2002; received in revised form 19 April 2002; accepted 22 April 2002

Abstract
Animal models that simulate various aspects of human brain aging are an essential step in the development of interventions to manage cognitive dysfunction in the elderly. Over the past several years we have been studying cognition and neuropathology in the aged-canine (dog). Like humans, canines naturally accumulate deposits of β-amyloid (Aβ) in the brain with age. Further, canines and humans share the same Aβ sequence and also first show deposits of the longer Aβ1–42 species followed by the deposition of Aβ1–40. Aged canines like humans also show increased oxidative damage. As a function of age, canines show impaired learning and memory on tasks similar to those used in aged primates and humans. The extent of Aβ deposition correlates with the severity of cognitive dysfunction in canines. To test the hypothesis that a cascade of mechanisms centered on oxidative damage and Aβ results in cognitive dysfunction we have evaluated the cognitive effects of an antioxidant diet in aged canines. The diet resulted in a significant improvement in the ability of aged but not young animals to acquire progressively more difficult learning tasks (e.g. oddity discrimination learning). The canine represent a higher animal model to study the earliest declines in the cognitive continuum that includes age associated memory impairments (AAMI) and mild cognitive impairment (MCI) observed in human aging. Thus, studies in the canine model suggest that oxidative damage impairs cognitive function and that antioxidant treatment can result in significant improvements, supporting the need for further human studies.

© 2002 Published by Elsevier Science Inc.

Keywords: Dog; β-Amyloid (Aβ); Mild cognitive impairment

1. Introduction

The brain progressively accumulates oxidative damage and other types of neuropathology that ultimately result in neuronal dysfunction and cognitive decline. A key challenge is to identify mechanisms underlying pathological aging and to develop therapeutics to prevent or slow disease progression. Animal models, including rodents and nonhuman primates, are critical to the success of this research. Over the past several years we have been investigating a novel animal model of human cognitive aging, the aged canine. The advantages of using canines to study brain aging includes the following: (1) canines share many of the same environmental conditions with humans; (2) canines can perform a sophisticated repertoire of complex cognitive behaviors; (3) the brain in aged canines shows many pathological changes common to humans; and (4) neuropathology is significantly associated with cognitive decline.

Our strategy has been to identify brain and behavioral changes that appear with age and to determine if interventions that target proposed underlying cellular pathological mechanisms can improve cognitive function. The proof of principle to determine whether a specific type of neuropathology contributes to cognitive dysfunction is to show that an intervention targeting the proposed mechanism improves function. Of necessity, studies in humans are primarily correlative but help to establish key pathological mechanisms amenable to manipulation. Over time these studies may lead to clinical trials but even if successful it is difficult to determine if the intervention has an effect on brain pathology. In the canine model it is feasible to test interventions and determine the effect they have on the brain. In this review we present an overview of the progress in characterizing the canine model and the effects of antioxidants on cognitive function. The review has three parts:
Fig. 1. Aβ is associated with neuronal membranes in the aged canine brain. (A) A confocal image illustrating punctate deposits of Aβ1-42 along the cell body and processes of a neuron. Arrows indicate punctate regions of concentrated Aβ. (B) The distribution of gold particles (n = 419) distributed across the neuronal membrane in an axis perpendicular to the dendritic plasma membrane. The peak of the curve represents the maximum particle density and indicates that Aβ is concentrated along the membranes rather than within the extracellular space. (C) An example of labeled dendritic membranes (arrowheads) used for the analysis in B. Bar = 0.3 μm.
(1) an overview of neuropathology in the aged canine brain; (2) the nature of cognitive dysfunction in the aged canine; and (3) recent results demonstrating the effectiveness of an antioxidant intervention in improving cognitive performance on select tasks that decline with age.

2. Neuropathological features of the aged canine brain

A critical issue is to identify neuropathology that has the greatest functional impact on cognitive decline. The canine brain exhibits several key features observed in the aged human brain. Many of these consistent features are associated with early pathology seen in normal human brain aging, in the brains of individuals with mild cognitive impairment (MCI) and in Alzheimer’s disease (AD) patients. In the canine, these features do not develop into the full-blown pathology associated with moderate or severe AD. Thus, the canine serves as a model for early stage pathology [37].

One of the first reports of age-associated neuropathology in canines was in 1914 describing abnormal pyramidal neuron sprouting [45]. In the 1950’s, other types of neuropathology were reported including “Alzheimer-like” senile plaques [9,21–23,39,79]. Aged canine brains display a number of morphological signatures similar to those observed in aged human brains including cortical atrophy [70], myelin degeneration in the white matter [24], the accumulation of degraded proteins [7], DNA damage [3,42] and a reduction in endogenous antioxidants [43].

Canines naturally accumulate Aβ in the brain with increasing age [16,19,34,66] (Fig. 1) and form a diffuse type of plaque. The amino acid sequence of canine Aβ is identical to that of human Aβ [39]. In addition, there is clear evidence that Aβ accumulation can also be seen in association with neurons at both the light and electron microscopic level [16,72]. Specifically, Aβ appears to be concentrated within microdomains on the plasma membrane identified by immunogold labeling (Fig. 1). These same microdomains also contain presenilin, which is thought to play a role in cleaving the amyloid precursor protein (APP) leading to Aβ production [37]. This membrane localization may cause early functional changes in neurons that may be detectable at the behavioral level of analysis.

Not all brain regions are equally vulnerable to Aβ pathology; pathology develops in the prefrontal cortex at an earlier age and more consistently than other cortical areas studied, such as the entorhinal or parietal cortex [34]. The occipital cortex accumulates Aβ at a much later age than these other brain regions. This pattern of Aβ accumulation with age in canines parallels that seen in humans [8]. Within the prefrontal cortex, Aβ first appears in deep cortical layers and at later ages, the superficial layers are increasingly affected [67]. In studies of over 150 dog brains, Aβ deposition has not been observed in layer 1 of cortex, which contrasts with clear evidence of Aβ distribution in this layer of the human brain. On the other hand, a diffuse band of Aβ is observed in the outer molecular layer of the canine hippocampus where plaques are also found in the AD brain.

Another common characteristic between canine and human Aβ is that the predominant species of Aβ is the longer, toxic fragment Aβ1–42 [18,57,80]. At later ages the shorter, more soluble, fragment Aβ1–40 accumulates in plaques and in blood vessel walls. As with human brain aging, Aβ accumulates within the blood vessel walls of the aged canine brain suggesting that the canine may be a useful model to study Aβ angiopathy [64,74,76,78].

Tangles identical to those seen in the human brain are rare in other species and dogs do not develop mature tangles characterized by paired helical filaments [4,20,29,68,79]. However, it is likely that early tangles are present in aged canine brain, since canine tau also becomes hyperphosphorylated as in aged human brain, but they do not mature into the full phenotype [36,44]. Tau phosphorylation, as detected by the AT8 antibody, increases in the aged brain and thus possibly some of the early features of tangles are present in the aged canine brain [60,77]. The canine provides an opportunity to study the role of Aβ pathology on cognition in the absence of overt tangle formation.

Thus, the rationale for using the canine model to understand the role of Aβ in human brain aging include but are not limited to the following: (1) Aβ is normally deposited with increasing age; (2) the distribution of Aβ as a function of age parallels that of humans; and (3) the sequence in which specific fragments of Aβ are deposited is similar and the protein itself is identical to the human. Further, since Aβ deposits remain diffuse in aged dog brain, the model is well-suited for studying early stage pathology of brain aging/Alzheimer’s disease prior to the appearance of other complex variables such as tangle formation.

3. Cognitive dysfunction in aged canines

The advanced learning ability of canines is well known, as evidenced by their use as guides for the blind and as military working dogs. Our research has focused on a single breed, beagles, because longevity varies widely with respect to breed as does the age of onset and extent of Aβ [6]. The average life span of a beagle is 13.6 years but animals that live up to 18 years have been observed [67]. Beagles over the age of 8 years are considered old based upon evidence for reduced cerebrovascular function after this age [50]. However, breed differences in lifespan are substantial and larger breeds typically have shorter lifespans [46].

Learning and memory can be tested systematically in dogs using tasks developed for use in nonhuman primates. In parallel with the human and primate literature, tasks are selected that are sensitive to the function of specific cortical circuits and/or brain regions. All testing is conducted using food rewards, which sufficiently motivate dogs to learn each task. The use of deprivation protocols, which are particularly stressful for aged animals, is unnecessary.
Two main conclusions have evolved from these studies: (1) detecting cognitive dysfunction depends on the cognitive processes engaged, the task used and the relative level of difficulty, and (2) variability in the cognitive abilities of dogs increases with age. Aged dogs are able to learn simple skills, on average, to the same extent as younger dogs [54]. However, individual aged dogs can show pronounced impairments. Simple associative learning, such as visual discrimination (learning that one of two objects covers a food reward), typically remains intact with age [27,32,52,54,78]. Significant impairment is seen, however, on more complex discrimination learning problems, such as size and oddity discrimination learning [32,55]. Similar age differences in visual discrimination learning have been reported in primates [73]. On the other hand, prefrontal-dependent tasks are consistently impaired in aged dogs [54]. One of these age-sensitive visual discrimination tasks is a reversal learning problem. Subsequent to successful attainment of a preset criterion level of response on a visual discrimination task the reward contingencies are reversed and animals must shift from responding to one object to the other. Reversal learning involves response inhibition and the ability to shift strategies, functions that are mediated by the prefrontal cortex [27,75].

In addition to learning ability, memory is also compromised in aged canines. Forms of memory that appear to be age-sensitive include spatial memory (the ability to remember the location of a food reward) and object recognition memory (the ability to recognize an object seen 10–120 s previously) [1,12,35]. The variability in performance of these tasks, however, is extensive. Aged dogs can fall into one of three categories: (1) unimpaired or successful agers; (2) age-impaired; (3) severely impaired. These clusters of aged dogs may be analogous to normal aging, MCI and dementia in humans.

The decline in learning and memory in laboratory studies is also consistent with clinical features observed by veterinarians who have identified a canine cognitive dysfunction syndrome (CDS), based on informant-based questionnaires or checklists [13,55]. CDS is characterized by dogs showing signs in one or more categories that include disorientation, disruptions in activity and sleep, changes in house-training and alterations in interactions with family members. In a survey of 26 owners of aged dogs, common complaints were destructive behaviors, inappropriate urination or defecation and excessive vocalization in older animals. Data from a study at UC Davis Veterinary College involved interviews with owners of 180 dogs aged 11–16 years whose pets had no illnesses that would account for behavioral signs such as altered social interaction with owners, sleep–wake cycles, and activity levels, housesoiling and disorientation. In this study, 28% of dogs between the ages of 11 and 12 and 68% of 15–16-year-olds were positive for at least one category. Ten percent of owners of 11–12-year-old dogs and 36% of owners of 15–16-year-old dogs had signs in two or more categories [58].

4. Relationship between age, pathology and behavior in aged canines

Is cognitive dysfunction associated with Aβ neuropathology? Several studies demonstrate a strong and significant association between the extent of Aβ deposition and the extent of cognitive dysfunction in dogs [16,17,32] similar to that reported in the human brain [15] (Fig. 2). This association can be further refined on a brain region basis: for example, Aβ in the prefrontal cortex is correlated with frontal-dependent learning and memory deficits [32]. A recent paper by Colle et al showed a significant association between behavioral dysfunction in aged dogs and the extent of Aβ deposition [53]. This recent publication, along with previous reports, supports an association between clinical measures of cognitive dysfunction and pathophysiology in aged canine brain.

While the accumulation of Aβ is part of a series of neuropathological events, it is unlikely to be the only contributing factor to cognitive decline. In our view, the basic molecular events in the aging brain form a cascade involving a sequence of feed-forward and feed-back mechanisms that culminate in neuronal dysfunction and Aβ deposition. Oxidative damage probably plays a central and pivotal role in the evolution of this cascade (Fig. 3).

5. Oxidative damage and brain aging

The brain has among the highest respiratory rate of any tissue and generates oxidative damage that progressively increases over time [2]. Neurons, are particularly vulnerable to cumulative oxidative damage because they are nondividing cells and survive for decades. The generation of oxidants leads to damage to proteins, lipids and nucleotides, which may contribute significantly to neuron dysfunction and degeneration associated with aging and neurodegenerative diseases [25,48]. Oxidative damage may serve as a common mechanism initiating and linking several pathological features of the aging brain. For example, the APP is vulnerable to oxidative damage and metabolic stress favors the production of amyloidogenic fragments [28,56]. Transgenic mice overexpressing mutant human APP (Tg2576) showed increased oxidative damage to lipids prior to overt Aβ deposition, which provides further evidence of oxidative damage being an early event [63]. Aβ is also able to directly generate oxidative damage to lipids and proteins [5,30,11]. According, to this model, antioxidants may have beneficial effects on brain aging at multiple stages.

Oxidative damage to lipids and proteins increase with age in the canine brain [33]. A significant increase in lipid peroxidation, measured by malondialdehyde (MDA) and damage to proteins, measured by carbonyl formation, was observed with age. A significant decline in glutamine synthetase activity, an enzyme vulnerable to oxidative damage and in the level of reduced glutathione (GSH) was observed...
Fig. 2. (A) Aβ immunostaining in the prefrontal cortex of an unimpaired 13-year-old beagle dog takes the form of diffuse senile plaques in layers III–VI. (B) A section from the frontal cortex of a 90-year-old female nondemented control case illustrating a similar pattern of senile plaque deposition as in the aged dog. Note that in both cases, Aβ is distributed in deeper cortical layers. (C) Aβ immunostaining in the prefrontal cortex of a severely impaired 12-year-old beagle dog is extensive and affects layers II–VI. The molecular layer is free of Aβ deposition (indicated by the vertical line). (D) For comparison, a sample of the frontal cortex from an 86-year-old male with Alzheimer’s disease shows a parallel extent of Aβ deposition as the dog. Note that diffuse senile plaques are similar in size between the dog and the human. On the other hand, dogs do not develop compact plaques (indicated by arrow in D). Bar = 200 μm.

Fig. 3. Oxidation causes damage to lipids, proteins and DNA/RNA. Oxidative stress also induces the expression of APP and can contribute to misprocessing of APP leading to generation of amyloidogenic fragments. The production of Aβ fragments may lead to a loss of compensatory ability (decreased bcl-2, increased bax). All of these factors in turn contribute to more Aβ deposition, possibly synapse loss and DNA damage. Ultimately, the pathways converge and result in neuron dysfunction and/or in some neurons death.

with age. MDA level in serum was a significant predictor of MDA accumulation in the prefrontal cortex (Fig. 4). Thus, the canine brain accumulates oxidative damage and in our model is an early event in the cascade. Establishing a link between oxidative damage, Aβ and cognitive function in the rodent brain is hindered by the lack of natural age-associated Aβ deposition. In human brain, studies are further complicated by the presence of neurofibrillary tangles. Unlike humans, aged canines develop extensive Aβ in the absence of neurofibrillary tangle formation [18]. The canine brain, therefore, is a simpler model for examining the association between age, oxidative damage, Aβ and cognitive function. Thus, studies in the canine model can complement studies in other animal model systems and provide further insights into human brain aging and neurodegenerative diseases.

6. An antioxidant diet improves learning in the aged canine

Accordingly, we have initiated a series of studies to test the hypothesis that an antioxidant diet can result in improvements in learning and memory and reduce the extent of pathology that accumulates in the aged brain [55]. We have collected extensive data in an ongoing study on learning and memory with treatment but results of the neuropathology
Fig. 4. Individual oxidative damage markers are plotted as a function of age in 19 beagle dogs. Progressive and significant increases in (A) brain malondialdehyde (MDA), and (B) protein carbonyl formation were observed. (C) Decreases in glutamine synthetase (GS) activity were found. (D) Individual MDA levels in the serum are plotted as a function of MDA levels in the prefrontal cortex. Higher serum levels of MDA are significantly correlated with higher prefrontal cortex levels of MDA.

studies are not available at present. The study is being conducted as a random placebo controlled clinical trial. The study involves the selection of animals by rigorous inclusion/exclusion criteria. Throughout the study, data is monitored by an external clinical trials coordinator.

Approximately, 1 year prior to the initiation of this study, old and young dogs were given a series of baseline cognitive tests, which were used to assign animals to cognitively equivalent groups. One of the aged groups and one of the young groups was subsequently changed to a food identical to the control but enriched with a broad spectrum of antioxidants and mitochondrial enzymatic cofactors; the other groups were maintained on the control food. The animals were maintained on the dietary intervention for approximately 6 months prior to scheduled cognitive assessment. The food was supplemented with Vitamins E and C; a mixture of fruits and vegetables, alpha-lipoic acid and L-carnitine (mitochondrial cofactors) to reduce oxidative damage to cells. These agents were selected on the basis of their mechanism of action and preliminary data examining these ingredients singly and in combination on measures of serum and urinary oxidative damage in dogs.

One of the tasks used was an oddity discrimination task, in which the animals were trained on a series of four increasingly more difficult learning problems. Each task involves repeatedly presenting three objects, two of which were identical, and one odd. Using progressively more similar objects for each new problem increases, the difficulty of the task. The animal receives a reward if it selected the odd object. This test protocol provides a series of learning problems of sufficient difficulty to show age sensitivity. The performance of monkeys trained on a similar task also varies as a function of the extent of similarity of the objects used [38,71].

In this task young animals are able to learn the series of tasks without showing a significant increase in error scores whereas the old animals generate additional errors as the task becomes more difficult. For the old animals, performance on the first task did not differ from performance on the second. All other task comparisons were statistically significant; Fig. 5 illustrates that these results
Fig. 5. Effect of age and diet on number of errors made in learning four progressively more difficult oddity discrimination tasks. Aged dogs learned each oddity task with significantly more errors than young dogs as can be seen by comparing young and old animals on the control diet. A diet enriched in antioxidants significantly improved learning in the two most difficult problems, oddity-3 and oddity-4 in aged dogs. Similar improvements were not found in the young dogs provided with the diet.

are due to the animals making more errors on each successively more difficult task than they had on the previous task \((P < 0.025)\). The young animals, by contrast, did not show significant differences in performance between any two tasks.

The results of the dietary manipulation are also shown in Fig. 5. The significant overall effect of diet was due exclusively to superior learning shown by the old animals on the antioxidant diet, when compared to the old animals on the control diet. The effect of dietary treatment also varied as a function of task. Diet did not significantly affect performance on oddity 1, the first and simpler task. On the second task, the interaction between age by diet was marginally significant, \(F(1, 35) = 3.904, P = 0.056\). On task 3, the diet effect was highly significant \(F(1, 34) = 12.32, P = 0.0013\) as was the diet by age interaction, \(F(1, 34) = 9.715, P = 0.004\). Task 4 also had a significant diet effect \(F(1, 34) = 4.78, P = 0.035\) and diet by age interaction, \(F(1, 34) = 5.118, P = 0.030\). Thus, the antioxidant diet produced an improvement in the ability of old dogs to learn a complex task.

The oddity discrimination task provides a sensitive measure of age-dependent cognitive deterioration in dogs, and this age-dependent effect can be at least partially reduced by maintenance on a food fortified with a complex mix of antioxidants and mitochondrial enzymatic cofactors. The use of a series of problems of graded difficulty is an essential design feature of the study and is not commonly used in assessing cognitive interventions in animal models. The protocol revealed that both age and diet effects are amplified by increasing the difficulty of the task. A single level of task difficulty may not have revealed clear effects because of the task being either too easy, or too difficult. Thus, we did not find a significant effect of diet on the first and easiest of the oddity discrimination problems. Similar
results were obtained on landmark discrimination learning, which tests spatial attention [53]. The most important result of this study was the superior performance of the aged animals on the enriched diet compared to controls. A number of factors probably account for the strong dietary effects, including use of aged subjects, 6 months or greater maintenance on the diet, use of a test protocol with progressively more complex problems, and the particular components of the diet. The possibility that the intervention leads to a general, age-independent, improvement in brain function can be excluded since the diet had minimal effects on the young dogs. Thus, oxidative damage is unlikely to induce substantial neuronal dysfunction until relatively late in life.

With respect to dietary constituents, to our knowledge, this is the first study to use combined substances that target enhancement of mitochondrial function with antioxidants that suppress the action of free radicals in a higher animal model. Our results build upon and extend the findings that antioxidants or mitochondrial cofactors alone decrease age related cognitive decline in other species [30,31,40,41,49,69,81]. Our results may be attributable to two different synergistic strategies; first, a complex mixture of antioxidants that supports a network of antioxidants requiring several components to act together for effective function, and; second, improved mitochondrial metabolic function that decreased free-radical production while improving mitochondrial energetics and efficiency.

Alternatively, a reduction in oxidative stress may retard various downstream mechanisms resulting in neuronal dysfunction. Many of the antioxidants utilized in this study also have anti-inflammatory properties [26,47,51]. There has been an association of non-steroidal anti-inflammatory intake and decreased incidence of dementia in humans, which suggests that inflammation is a contributor to neurocognitive decline [30]. As such, the antioxidants included in this dietary fortification may have acted via an anti-inflammatory path, or synergistically, with antioxidant mechanisms to elicit the profound cognitive effects observed.

7. Conclusions

Aged canines, like humans develop age-related neuropathologies, particularly the accumulation of Aβ, develop impaired cognitive function. We hypothesize that cognitive function in canines declines along a “cognitive continuum” that reflects a similar phenomenon in humans [61]. In humans, the continuum is postulated to begin with the development of age associated memory impairment (AAMI) defined as a loss in memory on one or more tests that is 1 S.D. below that of the young population normative values [14]. Probably, because the presence of AAMI is so prevalent in the population of elderly individuals (estimated to be over 50%), the risk for conversion to more advanced stages is relatively low. AAMI is followed by MCI defined by a decline in one or memory functions that is greater than 1.5 S.D. below age-matched norms but is associated with normal activities of daily living [62]. MCI increases the risk for conversion into dementia, particularly AD. Dementia reflects deficits in multiple cognitive domains and the loss of normal activities of daily living. In the aged canine population, the cognitive continuum is primarily associated with the earliest stages, AAMI or MCI though some canines will develop the equivalent of dementia. In the veterinary literature, this latter phase is generally classified as CDS defined as memory impairments and losses in activities of daily living (e.g. social interactions, grooming, disruption of sleep–wake cycles). These features are consistent with the milder expression of neuropathology in canines emphasizing oxidative damage, Aβ accumulation in the form of diffuse plaques and the early stages associated with tangle formation. Thus, the canine represents a higher animal model to study the earliest declines in the cognitive continuum observed in human aging.

We suggest that the combination of antioxidants with mitochondrial enzymatic cofactors may work together synergistically leading to an improvement in learning and memory associated with the progressive decline along the cognitive continuum. Taken together our data supports the hypothesis that oxidative damage and mitochondrial function is a fundamental mechanism contributing to age-associated cognitive dysfunction and underscores the need to conduct similar trials in humans.

Acknowledgments

This project was sponsored by funds provided by the National Institute of Aging (Grant AG12694), the US Department of the Army, Contract No. DAMD17-98-1-8622, and by Hill’s Pet Nutrition, Inc. Topeka, KS. The content of the information does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred. The investigators adhered to the “Guide for the Care and Use of Laboratory Animals” prepared by the Committee on Care and Use of Laboratory Animals of the Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985). Research was conducted in facilities fully accredited by the AAALACI. Additional funding was provided by Science & Technology Center, Hill’s Pet Nutrition, Inc., P.O. Box 1658, Topeka, KS. We thank Heather Murphy and Candace Ikeda-Douglas for their assistance in obtaining cognitive data from the longitudinal studies.

References


